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Chronic beryllium disease (CBD) is characterized by granulomatous inflammation and the accumulation of CD4+ T cells in the lung. Patch testing of CBD patients with beryllium sulfate results in granulomatous inflammation in the skin. We investigated whether the T cell clonal populations present in the lung of CBD patients would also be present in the involved skin of a positive beryllium patch test and thus mirror the granulomatous process in the lung. CBD patients with clonal TCR expansions in skin and BAL were as high as 40% in selected Vβ T cell subsets. Studies of peripheral blood T cells before and after patch testing provided evidence for mobilization of large numbers of pathogenic beryllium-reactive T cells into the circulating pool. These studies using skin patch testing provide new insight into the dynamics of T cell influx and mobilization during granulomatous inflammation. The Journal of Immunology, 2002, 168: 3627–3634.

C hronic beryllium disease (CBD) is a granulomatous disorder that develops in up to 16% of individuals exposed to beryllium, depending on the nature of the exposure and the genetic susceptibility of the individual (1–7). Exposure to beryllium continues to be a major public health concern because of its continued use in ceramics, aerospace, electronics, and defense industries (1). Although the lung is the predominant organ involved, other organ systems such as the skin, liver, and lymphatic system may also be affected by the granulomatous inflammatory response (8, 9). The current disease definition requires a history of beryllium exposure, the presence of granulomatous and/or mononuclear cell inflammation in a biopsy specimen, and a proliferative response of blood or lung T cells to beryllium salts in vitro (10–12). Susceptibility to CBD has been associated with particular alleles of the class II MHC molecule, HLA-DP (5–7, 13), and the mechanism for this susceptibility has been tied to the ability of these HLA alleles to present beryllium to specific CD4+ T cells (14, 15).

Considerable evidence suggests that CD4+ T cells are important in the immune response to beryllium and in the immunopathogenesis of CBD (11, 16–19). For example, the development of granulomatous inflammation is associated with the accumulation of CD4+ T cells in the bronchoalveolar lavage (BAL). In a number of individuals, these T cells included oligoclonal T cell expansions with particular TCRs specific for CBD (19). CD4+ T cells in the BAL of CBD patients have been shown to proliferate and release Th1 cytokines in vitro in the presence of APCs expressing class II MHC molecules (14, 15, 17). It is currently unknown whether the T cells present in BAL fluid reflect the same T cells that drive granulomatous inflammation in the tissue. Lung tissue is relatively inaccessible to repeated sampling or to large samples, as are many organs affected by immune-mediated disease. In addition, the CD4+ T cells in BAL have been studied only after many years of disease, after granulomas are well established. Finally, CD4+ T cell function has been assessed primarily by in vitro testing. An assay system that analyzed the in vivo T cell-dependent process in the development of granulomatous diseases would likely add important new insight into the disease process.

The immune basis of CBD was first suggested by the demonstration of a delayed-type hypersensitivity response in beryllium-exposed subjects following skin patch testing with beryllium salts (20, 21). However, the use of beryllium patch testing as a diagnostic tool decreased in popularity because of the potential risk of inducing sensitization and the theoretical risk that the underlying disease would be exacerbated (22). Recently, patch testing has been re-investigated as a diagnostic tool (23–25). In 14 subjects with CBD, positive patch tests were associated with no detectable adverse effects (25). In the present study, we used beryllium patch testing in CBD patients to compare the repertoire of T cells infiltrating skin with BAL T cells in the same individual. We noted that the CD4+ T cells infiltrating skin in response to beryllium varied depending on the timing and pathology. Early T cell infiltrates in the skin, before granuloma formation, expressed a different set of
TCRs. In contrast, later in the course of granuloma formation, a significant fraction of skin T cells matched those present in BAL and expressed beryllium-reactive TCRs previously identified as specific for CBD. These T cell specificities were also detected for the first time in the circulating pool. These studies demonstrate an in vivo model of granuloma formation in humans with mobilization and influx of pathogenic CD4+ T cells similar to that which occurs in the internal organ primarily affected by this disease.

Materials and Methods

Study population

The diagnosis of CBD was established using previously defined criteria (11, 26), including a history of beryllium exposure, the presence of granulomatous inflammation on lung biopsy, and a positive proliferative response of BAL T cells to beryllium sulfate (BeSO4) in vitro. We studied three CBD patients in whom CD4+ T cell expansions were previously identified in the BAL (two with TCR Vβ3- and one with TCR Vβ14-expressing expansions) (18). CBD patients 1 and 3 were being treated with corticosteroids at the time of enrollment in this study. Informed consent was obtained from each CBD patient, and the protocol was approved by the Human Subject Institutional Review Boards at the University of Colorado Health Sciences Center and National Jewish Medical and Research Center (Denver, CO).

BeSO4 patch testing of CBD patients

Patch testing was performed using a modified version of the methods of the North American Dermatitis Group (27). One percent aqueous BeSO4 in Finn chambers was applied to normal unabraded skin on the back for 48 h. The patch test was interpreted at 96 h as follows: 1+ weak (nonvesicular) positive reaction; 2+, strong (vesicular) positive reaction; 3+, extreme (bullous) positive reaction. A 3- or 4-mm punch biopsy was performed at day 0, 24 h, and 96 h after patch testing. Three of these individuals were selected to undergo BeSO4 patch testing of CBD patients 1, 2, and 3 per protocol. Patients 1 and 3 had previously shown positive skin tests for BeSO4. The diagnosis of CBD was established using previously defined criteria (11, 26).

In vitro stimulation

Peripheral blood lymphocytes were cultured in 96-well plates in a humidified incubator at 37°C with 5% CO2 and 95% air. PHA (final concentration 5 μg/ml) was used as a positive control in all experiments. Mitomycin C (final concentration 1 μg/ml) was used to eliminate spontaneous proliferation. BeSO4 was used at concentrations ranging from 10-4 M to 10-2 M. At 72 h, cells were harvested and processed for analysis as described below.

Immunohistochemistry of BeSO4 skin patch tests

A second punch biopsy was collected from each subject at the designated time points, fixed, and cut into 5-μm sections. Antis directed against CD3, CD4, and CD8 (PharMingen, San Diego, CA), and DAB (monoclonal IgG1; DAKO, Carpinteria, CA) were used for immunohistochemistry. Avi- din-biotin complex peroxidase methods were used for staining based on VectaStain Elite ABC kit (Vector Laboratories, Burlingame, CA). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide, then blocked with 1.5% normal horse serum for 30 min. Sections were incubated for 60 min with the primary Ab diluted in 1.5% normal horse serum, washed in PBS, and incubated for 30 min with biotinylated horse antimouse IgG Ab. Following washing, sections were incubated for 30 min with HRP-conjugated avidin-biotinylated complex, washed again with PBS, then developed with 3′-diaminobenzidine (Vector Laboratories) and counterstained with hematoxylin. All samples were also stained using H&E.

Isolation and analysis of TCR expression in peripheral blood, BAL, and skin biopsies

Mononuclear cells were isolated from heparinized blood by Ficoll-Hypaque (Amersham Pharmacia Biotech, Piscatway, NJ) density gradient separation and from BAL as previously described (28). Peripheral blood cells from CBD patients were stained with FITC-labeled anti-CD4 mAb (BD Biosciences), and CD4+ T cells were sorted using a MoFlo cell sorter (Cytomation, Fort Collins, CO). Sorting of BAL cells from CBD patients for CD4+ T cells was not performed, due to the >80% predominance of CD4+ T cells in this compartment. Mononuclear cells were analyzed by two-color immunofluorescence for CD4 and TCR V region expression using mAbs directed to 11 different TCR Vβ receptors and one TCR Vα receptor as previously described (18, 29). Anti-TCR mAbs were biotinylated and streptavidin-PE (Fisher Biotech, Pittsburgh, PA) was used as a second-step reagent for TCR staining. Cells were also double-stained with FITC-labeled CD3, CD4, and CD8 mAbs (BD Biosciences). The lymphocyte population was identified using forward and 90° light scatter patterns, and fluorescence intensity was analyzed using a FACSscan cytometer (BD Biosciences) as previously described.

Analysis of TCRBV gene expression in peripheral blood, BAL, and skin biopsies

Total RNA was isolated using an acid guanidine-phenol chloroform method, and cDNA was synthesized using 2 μg RNA per 20-μl reaction. cDNA synthesis reagents included reverse transcriptase (SuperScript RT; Life Technologies, Grand Island, NY), RNase inhibitor (Promega, Madison, WI), and dNTPs and random hexamers (both from Amersham Pharmacia Biotech). One microliter of the cDNA reaction was added per 50 μl PCR mixture. PCR amplification (AmpliTaq; PerkinElmer, 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′ TCR β-chain gene (TCRB)V3 primer, 5′ TCRBV14 primer, 5′ TCRBV2 primer, and the 3′ CB primer were 5′-GTCCTCAGAGAAGAGAGG AGCGC-3′, 5′-GTCCTCTGAAAGAGAGAGAAT-3′, 5′-GCAACT TCCAAATGGAAGCTCC-3′, and 5′-TTCCTGATGCTCAAACAC-3′, respectively. The PCR products were ligated into the pCR II TA cloning vector (Invitrogen, San Diego, CA), and the ligation products were transformed into Epicurian Coli XL-1 Blue supercompetent Escherichia coli cells (Stratagene, 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′-CCCAGTCACGACGTTGTAAAACG-3′) sequencing primers and an automated ABI 377 sequencer (PE Applied Biosystems, Foster City, CA).

Lymphocyte proliferation assay

Proliferation assays were performed using PBMCs (2.5 × 10⁵ cells/well) cultured in 96-well flat-bottom microtiter plates in the presence of 1 μg/ml PHA for 48 h or BeSO4 at concentrations ranging from 1 × 10⁻⁴ M to 1 × 10⁻² M for 96 h. The wells were then pulsed with 1 μCi of [3H]thymidine for an additional 18 h, and incorporation of radioactivity was determined by beta emission spectroscopy. Proliferation assays were performed in triplicate.

Results

TCR expression in peripheral blood and BAL T cells of CBD patients

We have previously identified a subset of CBD patients with CD4+ T cell subset expansions in the BAL compared with blood (18, 19). Three of these individuals were selected to undergo BeSO4 patch testing in the current study and were restudied for alterations in TCR V region expression. The percentage of CD4+ T cells in the BAL and blood expressing particular V regions for patients 1–3 was determined by immunofluorescence staining and cytofluorographic analysis (Fig. 1). Patient 1 showed an increased percentage of Vβ3+ and Vβ13.1+ T cells in the BAL compared with the blood population (for Vβ3, 12.3% vs 6.1%, respectively). In patient 2, 9.3% of the CD4+ T cells in the BAL expressed Vβ3 as compared with 4.6% in the blood. Patient 3 demonstrated a Vβ14 expansion with 10.8% of the CD4+ T cells in the BAL expressing Vβ14 compared with 3% in the blood.

Histopathology of BeSO4 patch test skin biopsies

At 96 h after application of 1% BeSO4, all three individuals developed a strongly positive (2+) patch test reaction characterized by erythema, induration, and vesicles. No individual developed a positive reaction to normal saline. A separate study from this laboratory showed that control subjects with contact dermatitis and no history of beryllium exposure fail to develop a positive skin reaction following BeSO4 patch testing (25). Thus, patch testing of control subjects was not repeated in this study.

Skin biopsies obtained at 96 h after patch testing showed mild to moderate spongiosis involving the lower layers of the epidermis and focal edema of the papillary dermis (Fig. 2A). In contrast to the skin of healthy individuals, which usually harbors only a small number of lymphocytes, these biopsies showed a significant lymphocytic infiltrate in a perivascular distribution. There was no evidence of granulomatous inflammation at this early time point. Immunohistochemistry of the biopsy showed that the infiltrating...
lymphocytes expressed CD3 and CD4 (data not shown). Few CD8⁺ T cells were present.

Second skin biopsies of the same patch tests were done after ~2–5 wk. As shown in Fig. 2B, the spongiosis of the epidermis and the edema of the papillary dermis were resolved. Fig. 2C shows a poorly formed noncaseating granuloma. Staining of these lesions with anti-CD68 showed clusters of histiocytes and confirmed their granulomatous nature (data not shown). Similar to the findings at 96 h, the lymphocytic infiltrate was primarily composed of CD3⁺ cells (Fig. 2D) that expressed CD4 (Fig. 2E). Few, if any, CD8⁺ T cells were present.

Analysis of expressed TCRB genes in CBD patients

We focused on the TCR V regions expressed by expanded CD4⁺ T cell subsets in the BAL. Selection for skin CD4⁺ T cells was not performed due to the rarity of CD8⁺ T cells in the skin biopsies and the difficulty of obtaining adequate numbers of lymphocytes in a 3-mm punch biopsy specimen. BAL cells from CBD patients were also not sorted for CD4⁺ T cells due to the great predominance of CD4⁺ T cells in this compartment. However, we sorted CD4⁺ T cells from the blood of individual patients from which T cell clones in control samples (normal skin and skin following normal saline patch testing) and in the 96-h skin biopsy following BeSO₄ patch testing. However, none of these apparently expanded clonal populations in skin were identified in BAL, blood, or later samples of skin (Fig. 3), and none of the TCRs expressed by BAL expansions were detected in these skin samples (see below). In addition, no TCR sequence similarities were present among these different skin samples, e.g., normal skin (day 0) vs after saline or beryllium patch testing. The presence of clonal populations in normal skin and skin following normal saline patch testing in the face of no inflammation and few infiltrating lymphocytes could have been due to the very small amounts of TCR mRNA initially present and subsequent PCR amplification. Clonal expansions (i.e., two or more repeated sequences from the same T cell clone) were completely absent from the peripheral blood Vβ3⁺ CD4⁺ subset of patient 1.

In contrast to the early biopsies, T cells present in the skin later in the course of the BeSO₄ patch test reaction, at the time of granuloma formation, significantly overlapped with clonal populations in the BAL of the same individual (Fig. 4). For example, in patient 1, one TCRBV3 sequence (BV3-QGD-BJ1S5) accounted for 6 of 64 BV3 sequences (9.4%) in BAL and 2 of 39 (5.1%) in the skin (Fig. 4). This and other TCRBV3 sequences present in skin and/or BAL were noted to have a TCR complementarity-determining region (CDR)3 motif characteristic of pathogenic beryllium-reactive cells in CBD, and some sequences were identical to those previously described (19). This CDR3 motif includes an invariant aspartate (D) at position 96 of the β-chain and a length of 8 amino acids. One of the larger BAL clonal populations, BV3-PGGGLG-BJ2S4, accounted for ~16% of the BAL TCRBV3 repertoire and 5.1% of the skin TCRBV3 sequences. Overall, 21% of the skin TCRBV3 repertoire matched sequences present in BAL. Neither of these pathologic repertoires overlapped with sequences present in blood before patch test application. Similar to previous studies (19), peripheral blood T cells contained no clonal populations and no identifiable beryllium-reactive TCRs.

In the later biopsy of patient 3, 33% of the TCRBV14 sequences in skin were also present in BAL (Fig. 4). For example, one TCRBV14 sequence, BV14-KGGG-BJ1S5, accounted for 2 of 27 (7.4%) sequences in the skin and 3 of 37 (8.1%) sequences in the BAL. In addition, this sequence was also present in the blood CD4⁺ T cell population (1 of 35 sequences). The largest TCRBV14 clonal expansion in BAL was also present in the skin biopsy. In patient 3, we also examined the Vβ3 subset to see whether the similarity in skin and BAL repertoire after beryllium stimulation extended beyond the V regions expanded in BAL. The percentage of Vβ3⁺ CD4⁺ T cells in the BAL and blood of patient 3 were not different (4.1 and 5.2%, respectively) (Fig. 1). Similar to the findings for the expanded T cell subsets, TCRBV3 sequence matches were present (Fig. 4). For example, one TCRBV3 sequence, BV3-MRGGG-BJ2S2, accounted for 9 of 38 BV3 sequences (24%) in the lung and 5 of 26 (19%) in the skin. For this subset, nearly 31% of the skin sequences were present in the BAL repertoire and, conversely, 73% of the BAL sequences were present in the skin.

Mobilization of beryllium-reactive T cells into blood after patch testing

Previous studies have shown that beryllium-reactive T cells in the BAL of patients with CBD are rarely present in the circulating pool (18, 19). In the present work, we noted that this compartmentalization was upset after patch testing. TCRBV14, BV3, and BV2 sequences expressed by peripheral blood CD4⁺ T cells in patient 3 before and after BeSO₄ patch testing are shown in Fig. 5 (BAL sequences are shown again for reference). Before patch testing, 1 of 35 BV14, 1 of 18 BV3, and 0 of 28 BV2 sequences in blood
matched those present in BAL. Remarkably, at day 35 following patch testing, 63% of the blood TCRBV14 sequences were present in BAL and a similar percentage of BAL sequences were present in blood (Fig. 5). The largest BAL TCRBV14 clonal expansion, BV14-PKPTGVG-BJ2S6, was not detected in the blood CD4+ population initially, but following patch testing this sequence accounted for 22% of the TCRBV14 repertoire. For the BV3 subset, each of the three large clones in BAL were detected in blood after

**FIGURE 2.** Histology of representative skin punch biopsies following BeSO4 patch testing. A, H&E staining of a skin biopsy obtained 96 h after 1% aqueous BeSO4 patch test application. Mild to moderate spongiosis of the lower epidermis and edema in the papillary dermis are evident. A perivascular lymphocytic infiltrate is also apparent (original magnification, ×10). B, H&E staining of a skin biopsy obtained 35 days after BeSO4 patch test application. A decrease in the amount of papillary dermal edema (compared with 96 h) is seen. Lymphocytic infiltration in the dermis is also present (original magnification, ×10). C, Higher power magnification of a selected area of B showing a poorly formed noncaseating granuloma (original magnification, ×40). D, Expression of CD3 in an area of lymphocytic infiltration in a skin biopsy obtained 14 days after BeSO4 patch test application (original magnification, ×20). E, Expression of CD4 in the same biopsy as shown in C.

**FIGURE 3.** Expression of TCR Vβ3 gene (TCRBV3) amino acid sequences in peripheral blood, BAL, and skin T cells of patient 1. The sequence for the junctional region encompassing CDR3 is shown. Skin samples were obtained on day 0 (D0) before patch testing and 96 h and 14 days (D14) after patch testing with either saline or BeSO4. TCRBV3 sequences found at least two times are shown. The number of identical sequences (defined at the nucleotide level) is shown over the total number of sequences analyzed for a given anatomic site.
patch testing, but the extent of overlap between the blood and BAL repertoires was less than that seen for the BV14 subset. For the BV2 subset, none of the expanded BAL clones was detected in blood after patch testing.

In previous work, when BAL T cells were obtained from patients with CBD, in vitro stimulation with BeSO₄ caused a further expansion of the clones already present (18). This is consistent with the beryllium specificity of these clones. We observed the same phenomenon when peripheral blood T cells from patient 3 were obtained after patch testing and cultured for 5 days with BeSO₄ (Fig. 5). The frequency of TCRs matching in blood and BAL increased from 63 to 72% for BV14, from 9.4 to 53% for BV3, and from undetectable to 46% for BV2. The in vitro expansion of several BV3 and BV2 clones, initially identified in BAL, was dramatic.

The presence of these new sequences in the circulation suggested that functional responses of peripheral blood T cells to beryllium might be altered after patch testing. To test this possibility, PBMCs from patients 1 and 2 before and after patch testing were cultured in the presence BeSO₄ for 5 days (Fig. 6). Proliferative responses of cells from patient 1 were not detectable (stimulation index ≤2) before patch testing. These cells proliferated normally

![Image](https://www.jimmunol.org)
In response to PHA (data not shown). In contrast, when blood was obtained from patient 1 on day 14 after patch testing, proliferation to each concentration of BeSO₄ was apparent with stimulation indices as high as 14 at the previously determined optimal 1 × 10⁻³ M BeSO₄ (19). The extent of proliferation of cells from patient 2 also appeared to be significantly enhanced when obtained after patch testing, although the higher background proliferation resulted in very little change in stimulation indices. Peripheral blood cells from patient 3 obtained at day 35 following the BeSO₄ patch test proliferated vigorously in response to 1 × 10⁻³ M BeSO₄ (peak thymidine incorporation, 63,261 ± 2,156; stimulation index = 221) (data not shown in Fig. 6). Unfortunately, blood from day 0 was not available for comparison.

**Discussion**

Considerable evidence indicates that CBD is dependent on the generation and accumulation of beryllium-specific CD4⁺ T cells (17, 19). To study lung involvement, CD4⁺ T cells have been primarily obtained by washing the airspaces with saline and then collecting this fluid by aspiration, i.e., BAL. In patients with long-standing CBD, a population of beryllium-reactive CD4⁺ T cell clones are compartmentalized to this site (19). These cells recognize beryllium in the context of HLA-DP on APCs, and a subset is marked by oligoclonal expansions and particular beryllium-reactive TCRs (19). However, lung damage in CBD is secondary to the granulomatous inflammation in lung tissue, and it is currently unclear how the beryllium-reactive cells in BAL participate in the tissue inflammation. This issue has been difficult to assess because of the small size and random nature of transbronchial lung biopsies as well as difficulty in repeated sampling. Also, it is unknown whether the same T cells are involved in granulomatous inflammation at other non-lung sites and whether the compartmentalization to lung can be altered by a stimulus elsewhere. In the present study, we performed beryllium patch testing to address these issues. The results suggest that patch testing creates an in vivo model of the pathogenic granulomatous process. The development of granulomas in the skin was associated with mobilization and tissue infiltration of the same T cell clones present in BAL.

Few lymphocytes are present in the skin of healthy individuals. Within 96 h of applying BeSO₄ onto the skin of individuals sensitized to beryllium, inflammation accompanied by infiltration with CD4⁺ T cells occurs. Similar to previous studies (25), all individuals with CBD demonstrated erythema, induration, and a vesicular reaction typical of a contact dermatitis. This inflammation was beryllium specific in that it did not occur when saline was used instead of BeSO₄, and, although not tested in this study, inflammation has not been seen after beryllium patch testing of control individuals who do not have a history of beryllium exposure or evidence of beryllium-reactive T cells (25). Although clonal T cell populations were present in skin early after patch testing, none of the TCRs expressed by these cells matched those present in BAL of the same patients or was recognizable as beryllium-specific when compared with past studies (19, 30). Still, it seems likely that a subset of these early infiltrating cells were beryllium specific. It is possible that the few Ag-specific cells were masked by a larger number of nonspecific T cells. In the setting of an inflammatory response, the production of chemotactic factors directs migration of activated effector CD4⁺ T cells to sites of inflammation, regardless of specificity (31, 32). As a related example, mice immunized with myelin basic protein demonstrated minimal enrichment of pathogenic T cell subsets in inflammatory brain lesions (33).

Instead, T cells at the site of pathology expressed heterogeneous TCRs, with the pathologic T cell subset being obscured by the influx of nonspecific T cells (33). It is possible that culturing of the early skin T cells with IL-2 would have enriched for the subset of Ag-specific T cells as has been shown in studies of BAL (34) and sarcoidosis (35).

Our studies of early skin lesions also provide an important control for T cells present later in the granulomatous process. Thus, the subsequent appearance of repertoires enriched for BAL-like TCRs cannot be attributed to nonspecific recruitment of previously activated cells to sites of inflammation. Considering the enormous diversity of the T cell repertoire, finding T cells expressing identical TCRs in different target organs indicates selection by the same Ag, in this case beryllium. We were also able to identify TCRs documented to be beryllium specific based on previous studies of cloned Vβ3⁺ T cells from BAL. These TCRs are characterized by a β-chain CDR3 motif with an invariant aspartate (D) at position 96 and a particular length (19, 30). Although not analyzed in this study, the paired TCR α-chain is also highly related among the cells that express this beryllium specific β-chain. Although only one of the three patients in the current study had BAL T cells with this TCR, previous studies have demonstrated similar (or even identical) TCRs in almost all individuals with CBD caused by exposure to beryllium in the ceramics industry (30). Patients 2 and 3 in the current study were exposed in the nuclear weapons industry.

The presence and expansion of CD4⁺ T cells in skin that express TCRs identical to those in BAL provides new insight regarding the pathogenic potential of these cells. Infiltration into tissue in association with the granulomatous process strongly suggests that these CD4⁺ T cells do the same in lung tissue. Our studies also show that these cells have the capability of recognizing beryllium in other tissues, and it seems likely that granulomas in liver and lymphatic tissue in CBD involve the same subset of cells. It is currently not clear whether the pathogenic T cells arrive early in the skin and then expand over the next 2–5 wk or whether their arrival and expansion in the tissue are continuous during the generation of granulomas. The latter is supported by the high frequency of these cells in the circulating pool later after patch testing.

The granulomatous inflammation in CBD is identical to that in sarcoidosis, and these diseases share a number of clinical characteristics (36). The development of granulomatous inflammation in the skin following patch testing with BeSO₄ is similar to the Kveim-Siltzbach reaction seen in patients with sarcoidosis (37, 38).
Following the intradermal injection of the Kveim-Siltzbach reagent (a suspension of sarcoid tissue extract), noncaseating granulomatous inflammation develops after ~4 wk. Analysis of TCR β-chain repertoire of these reactions showed oligoclonal T cell populations consistent with a conventional Ag response (38). Compared with BAL T cells from sarcoidosis patients in general, similarities in the skin TCR CDR3 regions were noted to be suggestive, but analysis of BAL TCR repertoire in the same individuals was not performed and identical TCRs were not found. Our study is unique in this regard.

The majority of CBD patients will have blood cells that test positive in a beryllium lymphocyte proliferation assay (1). Thus, these individuals have beryllium-specific T cells in their circulating pool. Still, we have noted that there is a separation of lung and peripheral blood CD4+ TCR repertoires in these patients (19). Precursors or progeny of clonally expanded BAL T cells are absent or extremely rare in the circulating pool. The same was true in the present study, when blood T cells were analyzed before patch testing. These results likely relate to the low frequency of blood T cells that proliferate in response to beryllium in culture, which is below the frequency detectable by TCR sequence analysis. However, it is also possible that blood and BAL beryllium-specific T cells are separate populations with separate TCRs. In the present study, after 2–5 wk following patch testing, we noted a marked increase in T cells with TCRs identical to those expanded in BAL. The beryllium-specific nature of these cells is further supported by the enhanced proliferation of peripheral blood lymphocytes and the further expansion of particular T cell clones after in vitro stimulation with beryllium. Only considering the three TCRBV subsets (which account for ~20% of the repertoire), our results suggest that at least 2% of the peripheral blood CD4+ T cells may be beryllium specific after patch testing. This frequency of Ag-specific CD4+ T cells has only been reported for some patients with chronic viral infections (39). In contrast, memory CD4+ T cells specific for tetanus toxoid or purified protein derivative of Mycobacterium tuberculosis in healthy individuals occur at a frequency of 1 in 5,000 to 1 in 100,000 CD4 cells (40). Estimates of Th cell precursor frequency in the blood following injection of a conventional Ag like keyhole limpet hemocyanin ranged from 1 in 100,000 to 1 in 800,000 (41).

Our results show that application of BeSO4 to the skin of individuals with CBD results in a mobilization of beryllium-specific CD4+ T cells into the circulating pool. Depending on the degree of systemic Ag exposure after patch testing, the lymphoid tissue draining the patch test site or even the peri-lung lymphoid tissue may be the sites contributing to the circulating cells. The results prompted us to reevaluate the safety of patch testing in CBD patients. The safety of patch testing has been previously questioned (20, 21). More recent studies have found no evidence of clinical deterioration or progression related to patch testing (25). Additional follow-up of the same patients indicated no evidence of disease exacerbation (L. S. Newman, unpublished observation). Furthermore, no clinical changes have been noted in the three individuals patch tested in the current study, and follow-up is being continued. It should be noted that we deliberately selected study subjects for patch testing who no longer worked in the beryllium industry, as a further safeguard against the theoretical possibility of exacerbating the beryllium-induced immune response. However, the increased number of circulating beryllium-specific T cells after patch testing suggests the possibility that disease exacerbation may occur after patch testing, although none has been seen in the small number of patients followed to date.

Together, our results indicate that patch testing provides an in vivo model of granulomatous disease in individuals with CBD. We were able to observe the progression from influx of nonspecific T cells to the presence and expansion of beryllium-specific CD4+ T cells at the time of granuloma formation. The kinetics of this process cannot be studied in the organs of individuals with established disease and in vitro studies cannot provide the same insight shown in this work. Whether patch testing can be used in non-granulomatous diseases thought to be mediated by CD4+ T cells remains unknown. For example, patch testing may be useful to mobilize and characterize pathogenic CD4+ T cells in diseases where these cells are sequestered in target organs, such as the brain in multiple sclerosis, pancreas in insulin-dependent diabetes mellitus, or even joints in rheumatoid arthritis. Obviously, the safety of this procedure in these diseases would need to be addressed in a rigorous fashion.

References


