BIASED ACCUMULATION OF T LYMPHOCYTES WITH "MEMORY"-TYPE CD45 LEUKOCYTE COMMON ANTIGEN GENE EXPRESSION ON THE EPITHELIAL SURFACE OF THE HUMAN LUNG

By C. SALTINI, M. KIRBY, B. C. TRAPNELL, N. TAMURA, and R. G. CRYSTAL

From the Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

Although all T lymphocytes express antigen-specific receptors, there is increasing evidence that a population of blood T cells are "naive" in that they respond poorly to recall antigens, while other blood T lymphocytes are "memory" cells in that they proliferate readily in response to recall antigens, function efficiently as immune effector cells, and express surface molecules involved in antigen presentation and adhesion to endothelial cells and extracellular matrix components (1–5). The populations of "naive" and "memory" T cells are defined by the expression of the family of CD45 leukocyte common antigens coded by a single 130 kb, 33 exon gene (6). The CD45 gene uses differential splicing to generate multiple mRNA transcripts that direct the synthesis of 220, 205, 200, 190, and 180 kD glycoproteins (7–12). While the 220 and 205 kD glycoproteins are expressed on the surface of "naive" T cells and are detected by the 2H4 mAb, the 180 kD glycoprotein is expressed only on "memory" T cells and is detected by the UCHL1 antibody (11–14). Relative to the 33 exon CD45 gene, the mRNA transcript coding for the 220 kD $2H4^+$ protein contains the sequence information of all 33 exons, while the transcript coding for the 205 kD $2H4^+$ protein is missing residues coded by exon VI (12, 13). In contrast, the CD45 mRNA transcript coding for the 180 kD $UCHL1^+$ protein is missing exons IV, V, and VI (12–14).

A number of observations about T cells at sites of chronic inflammation suggest that the relative proportions of "naive" and "memory" T cell populations can vary within the body. In this regard, while blood T cells include both "naive" and "memory" T cells in approximately equal proportions, T cells participating in chronic inflammatory diseases in tissues are mostly comprised of "memory" T cells (15–18). The most likely explanation for the apparent compartmentalization of "naive" and "memory" T cells between tissues and blood is that the "memory" cells accumulate in inflamed tissues by selective margination, migration and/or proliferation in response to antigens at these sites (19, 20). If this is true, the same should hold in

C. Saltini's present address is Istituto di Tisiologia, Policlinico, University of Modena, Modena, Italy.

Address correspondence to Dr. R. G. Crystal, Pulmonary Branch, National Institutes of Health, Building 10, Room 6D03, Bethesda, MD 20892.

normal tissues chronically exposed to antigens, i.e., T cells present in such tissues should be biased toward relatively more "memory" cells than present in blood.

The present study was directed toward evaluating the relative proportions of "naive" and "memory" T cells on the epithelial surface of the normal lower respiratory tract, a site constantly exposed to environmental antigens (21). With the knowledge that "memory" T cells accumulate in areas of chronic inflammation (15–19), we hypothesized that the population of T cells on the epithelial surface of the normal lung should be dominated by "memory" T cells. As a control, the observations in normal lung were compared with the T cell populations on the epithelial surface of the lower respiratory tract in chronic beryllium disease, a chronic T cell–mediated inflammatory lung disease caused by an exaggerated response to a specific antigen, beryllium (22–24). Evaluation of $2H4^+$ and $UCHL1^+$ surface antigen expression by flow cytometry, together with quantitative analysis of specific forms of CD45 mRNA transcripts, demonstrates that the T cell populations on the epithelial surface of the lung in both groups of individuals are dominated by T lymphocytes expressing the UCHL1 surface glycoprotein and containing CD45 mRNA transcripts missing exons IV, V, and VI. Thus, the T cells on both normal and chronically inflamed lung epithelial surface are dominated by "memory" type cells.

Materials and Methods

The study population included 10 normal nonsmokers (age 32 ± 3 yr); all had normal chest x rays, and pulmonary function, and none had history of pulmonary disease. The population of individuals with chronic beryllium disease included 8 individuals (6 nonsmokers and 2 smokers; age 39 ± 3 yr), in whom the diagnosis was established using standard criteria (22–24). None of the patients were taking any medications at the time of evaluation. All data are presented as mean ± SEM, and all statistical comparisons were made using the two-tailed Student's *t*-test or the paired *t*-test, as appropriate. Logarithmic transformation was used to reduce the inequality of variance (25).

Source of T Lymphocytes. Blood mononuclear cells were obtained by venipuncture, and lung mononuclear cells were obtained by bronchoalveolar lavage (26). The volume of bronchoalveolar lavage fluid recovered in the normals was 57 ± 2% of that infused and 59 ± 2% in individuals with chronic beryllium disease. The amount of epithelial lining fluid (ELF)[1] recovered was quantified by the urea method (27). For the normals, 25 ± 3 × 10^3 cells/μl ELF were recovered, including 78 ± 6% alveolar macrophages, 17 ± 4% lymphocytes, 1 ± 1% neutrophils, and 0% eosinophils. For the individuals with chronic beryllium disease, the total recovery was 53 ± 25 × $10^3/\mu$l ELF, including 34 ± 5% alveolar macrophages, 66 ± 6% lymphocytes, 1 ± 1% neutrophils and 1 ± 1% eosinophils. Blood and lung T cells were purified using nylon wool columns (28); the resulting purity as assessed by the pan T cell antibody Leu5 (CD2; Becton Dickinson & Co., Mountain View, CA) was always >85% (see below for details of analysis). T cell subsets were purified by FACS (see below).

Immunofluorescence, Flow Cytometry, and Cell Sorting. The phenotype of lung and blood T cells was determined by immunofluorescence and flow cytometry (FACS IV; Becton Dickinson & Co.) as previously described (28) using the unconjugated, FITC- or phycoerythrin (PE)-conjugated mAbs Leu4 (CD3, T cells; Becton Dickinson & Co.), Leu5 (CD2, T cells; Becton Dickinson & Co.), Leu3 (CD4, helper/inducer T cells; Becton Dickinson & Co.), Leu2 (CD8, suppressor/cytotoxic T cells; Becton Dickinson & Co.), 2H4 (detecting the CD45 205, 220 kD proteins [12, 29, 30]; Coulter Immunology, Hialeah, FL), UCHL1 (detecting the CD45 180 kD protein [12, 14]; Dako Corp., Santa Barbara, CA). Isotype-matched unconju-

[1] *Abbreviations used in this paper:* ELF, epithelial lining fluid; PCR, polymerase chain reaction; PE, phycoerythrin; RT, reverse transcriptase.

gated, FITC- or PE-conjugated IgG (Becton Dickinson & Co.) were used as controls. To determine the proportions of lung and blood T cells expressing CD45 205, 220 kD and CD45 180 kD surface proteins, T cells were double stained with Leu4-PE and 2H4 or UCHL1 in conjunction with a FITC-conjugated goat anti-mouse antibody (Becton Dickinson & Co.) and analyzed with the FACS IV as previously described (28). Distributions of 2H4 and UCHL1 fluorescence intensity on blood $CD3^+$ T cells were analyzed on FACS fluorescence histograms using a curve-fitting program (COTFIT; B. Murphy, Carnegie-Mellon University, Pittsburgh, PA) to determine the lower confidence limit (mean ± 2 SD) of the $2H4^+$ or $UCHL1^+$ peaks; using this parameter, the percentages of "bright" $2H4^+$ (cells with a specific fluorescence intensity higher than the lower confidence limit) and bright $UCHL1^+$ T cells were quantified in blood and lung.

Blood T cell subsets were purified by FACS into $CD2^+2H4^+$ T cells and $CD2^+2H4^-$ T cell populations using the FACS IV as previously described (28). The purity of the resulting T cell populations was assessed by analysis of an aliquot of the sorted cells ($2H4^+$ cells: >99% $Leu5^+$, and >98% $2H4^+$; $2H4^+$ T cells: >99% $Leu5^+$, and <1% $2H4^+$).

Polymerase Chain Reaction (PCR) Amplification and Southern Analysis of CD45 mRNA Transcripts. The various species of CD45 mRNA transcripts in blood and lung T cells generated by alternate splicing of the primary transcription product of the CD45 gene were evaluated by obtaining mRNA from purified T cells and T cell subpopulations, converting the mRNA to cDNA with reverse transcriptase (RT), and then amplifying the specific cDNAs using PCR (31, 32) and CD45-specific oligonucleotide primers. To accomplish this, RNA was extracted from the T cells using guanidine isothiocyanate cell lysis and purified by cesium chloride gradient centrifugation (33). When the number of cells was limited to 10^6, RNA was extracted by NP40 cell lysis in the presence of ribonuclease inhibitor (RNAsin, 0.2 U/μl; Promega Biotec, Madison, WI) followed by enzymatic digestion of contaminating DNA (DNAse 5 U/ml; RQ1 Promega Biotec) and phenol/chloroform extraction in the presence of 150 μg/ml carrier tRNA (Bethesda Research Laboratories, Gaithersburg, MD) (34).

To convert T cell mRNA into cDNA, single-stranded DNA complementary to CD45 exons I through X was synthesized by primer extension using a CD45 exon X-specific oligonucleotide primer ("CD45 X," 5′ ATGTAACTGAAACTTTTC 3′; see Fig. 1) and AMV reverse transcriptase (Boehringer Mannheim Biochemicals, Indianapolis, IN) in 20 μl of RT buffer (50 mM Tris pH 8.3, 8 mM MgCl, 30 mM KCl, 0.3 mM dithiothreitol). All oligonucleotides were synthesized using an automated DNA synthesizer (Applied Biosystems, Inc., Foster City, CA) according to protocols supplied by the manufacturer and purified using Sephadex G 25 chromatography (PD10; Pharmacia Fine Chemicals, Piscataway, NJ). To amplify CD45-specific derived cDNAs, primers were used that would amplify all cDNA fragments derived from the differentially spliced CD45 mRNA transcripts. These CD45-specific primers included the 5′ primer "CD45 II" that annealed to exon II (5′ ATGTATTTGTGGCTTAAACTCTTG 3′) and the 3′ primer "CD45 IX" that annealed to exon IX (5′ GCAGTACATGAATTATGAGATATGG 3′; see Fig. 1 for location of primers). Because all CD45 transcripts are amplified by these primers, DNA products of variable size (376–870 bp) representing all different CD45 mRNA species should be obtained (Fig. 1). Since all T cells express CD45 gene products (1–5), this strategy allowed amplification of all CD45 mRNA species from all T cells, permitting quantitative evaluation of the expression of exon IV-positive CD45 mRNA ($CD45R\text{-}IV/2H4^+$) and exon IV, V, VI-negative CD45 mRNA ($CD45R\text{-}0/UCHL1^+$) transcripts using probes specific for each set of transcripts.

To accomplish this, PCR was performed using Taq polymerase (GeneAmp; Cetus Corp., Emeryville, CA) in combination with the CD45-specific primers (2.5 μM each) under standard conditions using the Thermal cycler (Perkin Elmer-Cetus) for 10 cycles (35). A fixed aliquot (5%) of the PCR product was then further amplified for 30 cycles. Eight different CD45 mRNA species are generated by the alternate splicing of the primary gene transcript (6–13). In order to identify the three PCR-amplified DNA fragments corresponding to the mRNAs coding for the $2H4^+$ and the $UCHL\text{-}1^+$ proteins, the PCR products were evaluated by Southern blotting analysis using a panel of four exon-specific genomic probes and exon-exon junction-specific synthetic oligonucleotide probes. The PCR products (10% of the reaction medium) were size fractionated on 2.5% agarose gels (1.5% NuSieve agarose

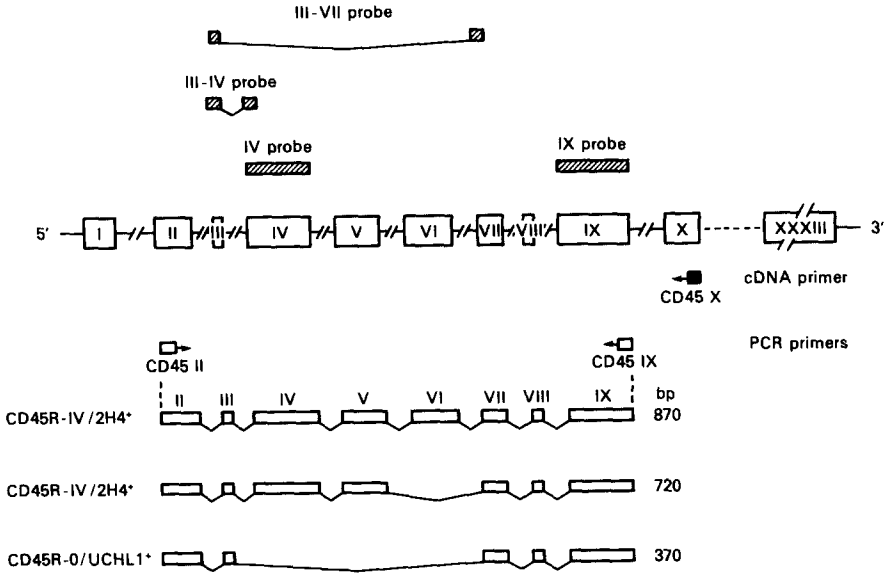

FIGURE 1. Schematic of the CD45 gene showing details of the PCR analysis used to evaluate the types of CD45 mRNA transcripts in blood and lung T cells. The CD45 gene contains 33 exons (6). Shown are the 5' region encompassing exons I–X, and the 3' exon XXXIII; only exons IV-VI are involved in alternative splicing (7–11). Below the gene is indicated the CD45-specific oligonucleotide primer used for cDNA synthesis ("CD45-X" cDNA primer; complementary to exon X) and the 5' and 3' primers used for PCR amplification (5' primer "CD45II" and 3' primer "CD45IX;" complementary to exon II and IX, respectively). Below the primers are shown the expected PCR products amplified from CD45R-IV/2H4$^+$ mRNA transcripts (870 and 720 bp) and CD45R-0/UCHL1$^+$ mRNA transcripts (370 bp). Shown above the gene are the DNA probes used for Southern analysis of PCR-amplified CD45 mRNA, including the "III-VII probe" to detect CD45R-0/UCHL1$^+$ mRNA transcripts; "III-IV probe" to detect CD45R-IV/2H4$^+$ transcripts; "IV probe" to detect CD45R-IV/2H4$^+$ transcripts; and a "IX probe" to detect all CD45 mRNA transcripts.

[Polysciences, Inc., Warrington, PA), 1% agarose, 1× TBE buffer [89 mM Tris-HCl, pH 8.3, 89 mM boric acid, 2 mM EDTA]) and transferred to nylon membranes (Zetaprobe; Biorad Laboratories, Richmond, CA) using 0.4 M NaOH (36) for hybridization with specific probes. The CD45-specific probes included (see Fig. 1 for location): (*a*) probe IX, pan CD45-specific exon IX, which is not spliced in any CD45 mRNA species (Eco RI/Hind III fragment of the genomic DNA clone pSP6.LCA.204C; M. Streuli, Dana Farber Cancer Institute, Boston, MA [11]); (*b*) probe IV, a probe encompassing exon IV specifically identifying CD45 mRNA species comprising exon IV (CD45R-IV) coding for the 2H4$^+$ gene product (11–13) (Bgl II/Bgl II fragment of the genomic DNA clone pSP6.LCA204A; M. Streuli [11]); (*c*) "probe III-IV," a probe encompassing the junction of exons III to IV, and thus CD45R-IV-specific (a synthetic 18-mer [5' TCCCCCACTGGATTGACT 3'] that is complementary to the exon III to exon IV junction sequence of the 2H4$^+$ cDNA clone LCA.6 [11, 13]); and (*d*) "probe III-VII," a probe encompassing the exon III to exon VII junction and thus specific for the mRNA species generated by complete splicing of exons IV, V, VI (CD45R-0) coding for the UCHL1$^+$ gene product (11) (a synthetic 18-mer [5' TCCCCCACTGATGCCTAC 3'] complementary to exon III-VII junction sequence of the UCHL1$^+$ CD45 cDNA clone LCA.1 [11, 12]). The genomic probes were labeled with [^{32}P]CTP (200 μCi/μg DNA) by nick translation (37). Oligonucleotide probes were end-labeled with [^{32}P]ATP (400 μCi/μg DNA) and T4 polynucleotide kinase (Bethesda Research Laboratories). The probes were purified

by Sephadex G 25 chromatography (37). Filter hybridization and autoradiography were performed using standard methods (36).

To validate the ability of these methods to quantify the levels of CD45-IV mRNA in blood and lung T cells, T cell RNA (CD45R-IV$^+$) was titrated in 20 μl of RT buffer, converted to cDNA, and amplified as above using PCR. Quantification was obtained with Southern analysis using the "probe IV" and laser densitometry.

To further evaluate whether the normal lung T cell populations are dominated by T cells expressing the CD45 gene in a fashion consistent with their UCHL1$^+$ surface phenotype (see Results), lung T cells were separated into single cells using the FACS IV equipped with a single cell deposition device (Becton Dickinson & Co.) (38), with 8.26 μm beads (Polybeads; Polysciences, Inc.) sorted on glass slides as a control. The cells were denatured (95°C, 5 min), the RNA was converted to cDNA (39), and the various CD45$^+$ mRNA transcripts were analyzed using specific primers as described above.

Results

Expression of CD45 Surface Proteins by Normal Blood and Lung Cells. Evaluation of cell surface expression of CD45 proteins by normal blood and lung T cells with the antibodies 2H4 and UCHL1 demonstrated that UCHL1$^+$ "memory" T cells selectively compartmentalize to the epithelial surface of the lower respiratory tract (Figs. 2, 3). While the relative proportions of blood T cells of the "naive" 2H4$^+$ and the

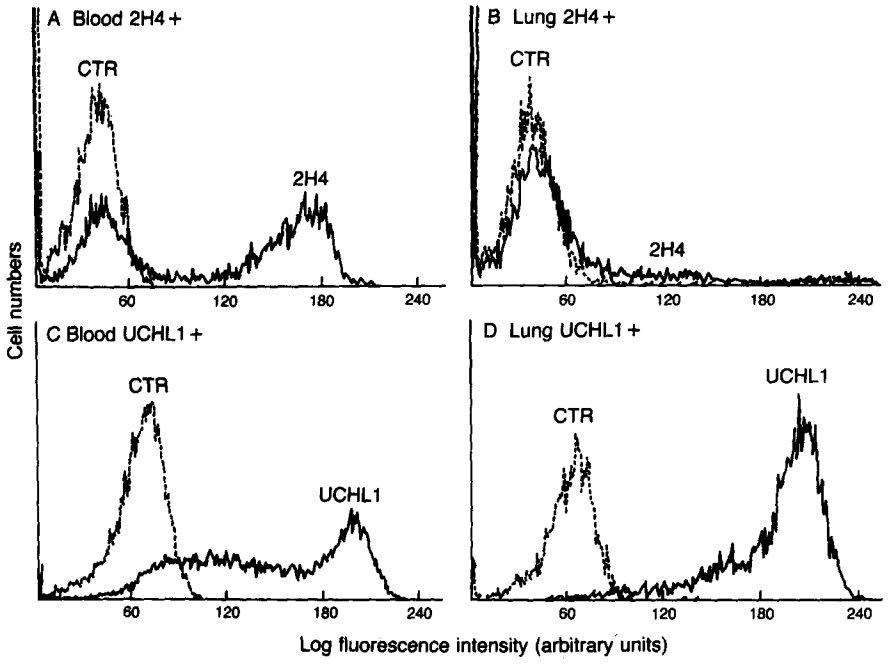

FIGURE 2. Flow cytometric analysis of normal blood and lung T cell surface expression of the 220 and 205 kD (antibody 2H4) and of the 180 kD (antibody UCHL1) CD45 proteins. The analysis for 2H4$^+$ cells and UCHL1$^+$ cells was carried out using two-color fluorescence together with the antibody Leu4 (CD3) to identify T cells. Shown are examples of 2H4 and UCHL1 fluorescence histograms of T cells in the CD3$^+$ gate. Abscissa: log fluorescence intensity. Ordinate: cell number. (*A*) 2H4$^+$ blood T cells. (*B*) 2H4$^+$ lung T cells. (*C*) UCHL1$^+$ blood T cells. (*D*) UCHL1$^+$ lung T cells. In each panel is shown the histogram with a control antibody (CTR); the vertical arrows indicate the region chosen to separate positive and negative cells (see Materials and Methods).

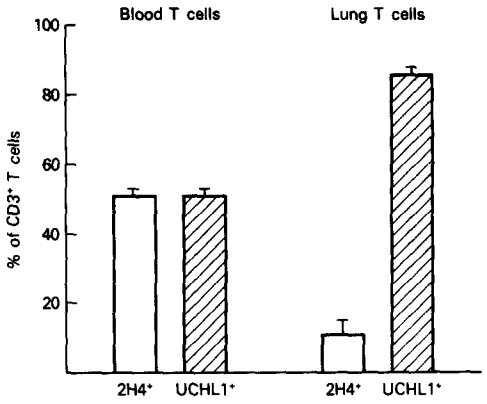

FIGURE 3. Flow cytometric evaluation of the proportions of $CD3^+$ T cells expressing the CD45 $2H4^+$ "naive" T cell phenotype or the CD45 $UCHL1^+$ "memory" T cell phenotype in blood and lung of normal individuals. The analysis was carried out as described in Fig. 2. Data are presented as the proportions of $CD3^+$ T cells ($Leu4^+$) that are also $2H4^+$ or $UCHL1^+$.

"memory" $UCHL1^+$ phenotypes are similar ($p > 0.1$), the lung T cell population was dominated by "memory" $UCHL1^+$ T cells, in striking excess of the $2H4^+$ T cells ($p < 0.01$).

CD45 mRNA Expression by 2H4 and 2H4 Blood T Cell Subpopulations. To analyze the expression of the CD45 mRNA forms by different T cell populations, PCR amplification of CD45 mRNA was used in conjunction with Southern analysis with a set of four genomic and oligonucleotide DNA probes that allowed specific identification of the mRNA transcripts coding for the CD45R-IV/$2H4^+$ CD45 product and the CD45R-0/$UCHL1^+$ CD45 product (see Fig. 1 for location of the probes and expected sizes of amplified products). Evaluation of normal blood T cell RNA with "probe IX," and exon IX-specific probe, identified several PCR products (ranging in size from 370 to 870 bp) corresponding to the various human CD45 mRNAs (at least five [7, 11]) generated by alternate splicing of the primary CD45 gene transcript. Among them were identified the CD45R-IV mRNA species coding for the $2H4^+$ proteins (870 and 720 bp PCR products) as well as the CD45R-0 mRNA species coding for the $UCHL1^+$ protein (370 bp PCR product; Fig. 4, lane *1*). "Probe IV," an exon IV-specific probe identified the CD45R-IV mRNAs (870 and 720 bp PCR products) but not the CD45R-0^+ 370 bp product (lane *2*). "Probe III-IV," an exon III-IV junction-specific probe, also identified the CD45R-IV^+ 870 and 720 bp products (lane *3*). In contrast, "probe III-VII," an exon III-VII junction-specific, probe identified only the CD45R-0^+ 370 bp product (lane *4*).

To demonstrate that differential T cell CD45 expression in "naive" and "memory" T cells at the mRNA level correlates with the $2H4^+$ and $UCHL1^+$ surface protein phenotypes, respectively, CD45R-IV^+ and CD45R-0^+ mRNA levels were evaluated in purified $2H4^+$ T cells and $2H4^-$ purified T cells (Fig. 5). Analysis of purified normal blood $CD3^+2H4^+$ T cells (Fig. 5 *A*) showed that they specifically expressed CD45R-IV mRNA (870 and 720 bp products [lane *1*]), but no CD45R-0 mRNA transcripts (370 bp product [lane *2*]). In contrast, $CD3^+2H4^-$ T cells (Fig. 5 *B*) do not express CD45R-IV (870 and 720 bp products [lane *3*]), but did express CD45R-0 transcripts (370 bp [lane *4*]). These observations are consistent with the concept that the specific CD45 RNA splicing patterns are different in the "naive" and "mem-

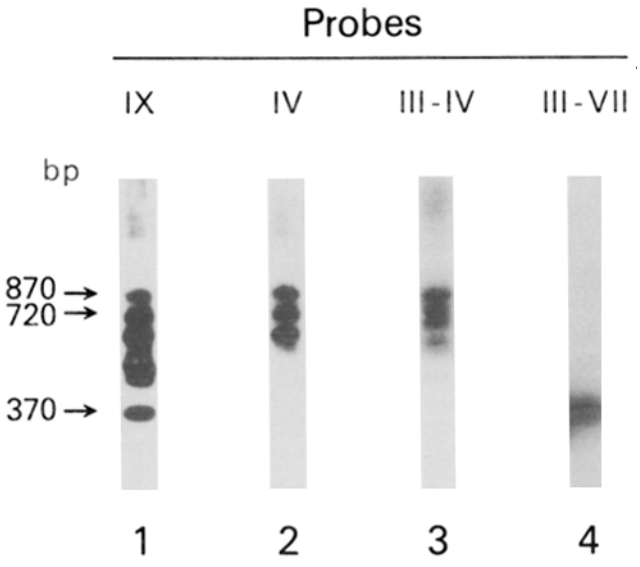

FIGURE 4. Examples of Southern analysis of PCR-amplified blood T cell CD45 mRNA transcripts, T cell RNA was converted to cDNA, amplified using 5′ CD45-II and 3′ CD45-IX primers (see Fig. 1), and analyzed using ^{32}P-labeled probes as indicated for each lane. (Lane *1*) Analysis with the "IX probe." The 870 and 720 bp products corresponding to CD45R-IV mRNAs coding for the 220 and 205 kD 2H4$^+$ proteins, respectively, are indicated, as are the 370 bp products corresponding to the CD45R-0 mRNA coding for the 180 kD UCHL1$^+$ CD45 protein. (Lane *2*) Analysis with the "IV probe." The 870 and 720 bp CD45R-IV$^+$ products are detected. (Lane *3*) Analysis with the "III-IV probe," showing the 870 and 720 bp CD45R-IV$^+$ transcripts. (Lane *4*) Analysis with the "III-VII probe," indicating CD45R-0$^+$ 370 bp transcripts.

ory" T cell populations, and correlate directly with the CD45 220, 205, and the 180 kD surface proteins that identify two nonoverlapping populations of T cells (1, 5).

Quantitative Evaluation of CD45 Expression in Normal Blood and Lung T Cells. The accuracy of the quantification of CD45R-IV$^+$ mRNA levels by PCR amplification was evaluated by preparing mixtures of a fixed amount of lung T cell mRNA (containing very little CD45R-IV mRNA) with increasing amounts of blood T cell mRNA (containing approximately equal amounts of CD45R-IV and CD45R-0 mRNA). Analysis of the amounts of PCR-amplified CD45R-IV$^+$ specific products demonstrated: (*a*) the methodology used could accurately quantify the amounts of specific CD45 mRNA transcript (correlation of input mRNA to specific output mRNA $r = 0.99$); and (*b*) the method was very sensitive, with as few as 1% of 2H4$^+$ T cells expressing CD45R-IV mRNA detectable (Fig. 6).

Using this approach, PCR analysis of blood and lung T cell CD45 mRNA levels using exon IX- and exon IV-specific CD45 probes demonstrated that in contrast to blood where the two types of CD45 mRNA patterns are equally expressed, T cells in the normal lung are dominated by T cells expressing the CD45R-0 "memory" T cell-type mRNA transcripts (Fig. 7). Strikingly, while blood T cells expressed the CD45R-IV/2H4$^+$ mRNA transcripts (870 and 720 bp CD45R-IV$^+$ fragments; Fig. 7 *A*, lane *1*), very little of this mRNA was present in lung T cells (lane *2*). Quantitative assessment of these transcript levels demonstrated the blood T cells contained, on the average, 17-fold more CD45R-IV mRNA per cell than did autologous lung T cells (Fig. 7 *A*, *right*; blood 35 ± 13 density units, lung 2 ± 1 density units $p < 0.01$). In contrast, both blood and lung T cells expressed the CD45R-0/UCHL1$^+$

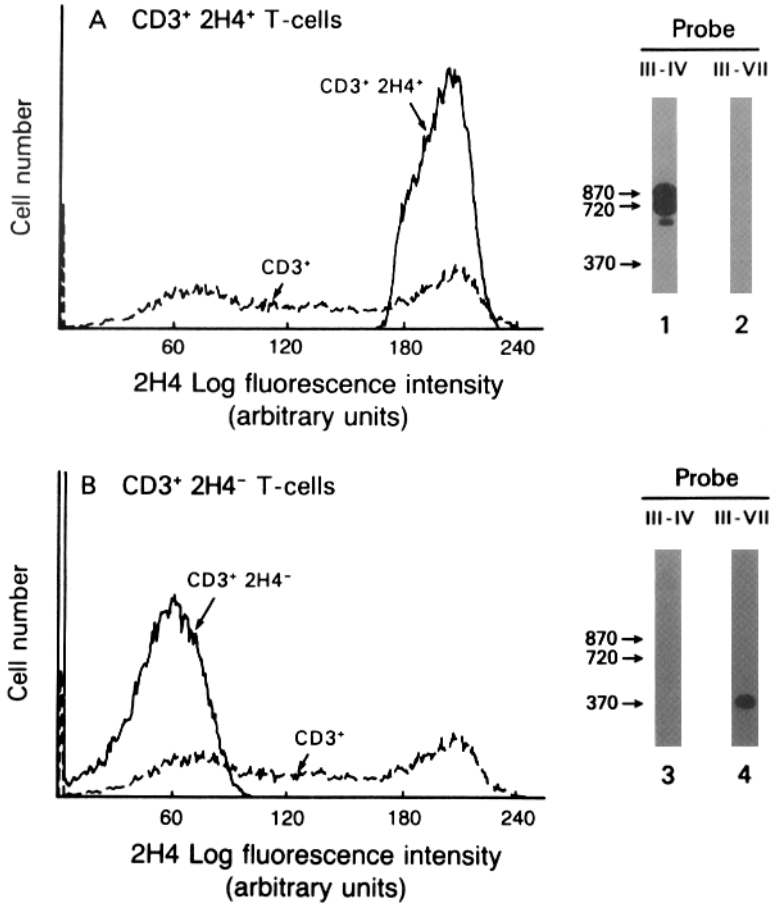

FIGURE 5. Examples of PCR analysis of CD45 mRNA transcripts expressed by purified blood $CD3^+2H4^+$ "naive" T cells and $CD3^+2H4^-$ "memory" T cell subpopulations. (*A*) *Left*, fluorescence histogram of cell sorter purified $CD3^+2H4^+$ T-cells (*solid line*). The profile of the unfractionated $CD3^+$ T cell population is shown as a dotted line. Abscissa: $2H4^+$ log fluorescence intensity. Ordinate: cell number. *Right*, Southern analysis of PCR-amplified mRNA transcripts from the purified $CD3^+2H4^+$ T cells. The probes were ^{32}P-labeled. (Lane *1*) Analysis with "III-IV probe." (Lane *2*) Analysis with the "III-VII probe." Indicated are the 870 and 720 bp products indicative of the CD45R-IV/$2H4^+$ transcripts. No 370 bp CD45R-0^+ product was observed. (*B*) *Left*, fluorescence histogram of cell sorter-purified $CD3^+2H4^-$ T cells (*solid line*). The profile of the unfractionated $CD3^+$ T cell population is shown as a dotted line. Abscissa: $2H4^+$ log fluorescence intensity. Ordinate: cell number. *Right*, Southern analysis of PCR amplified mRNA transcripts from the purified $CD3^+2H4^-$ T cells. (Lane *3*) Analysis with the "III-IV probe." (Lane *4*) Analysis with the "III-VII probe." Indicated is the 370 bp product indicative of CD45R-0/ $UCHLI^+$ transcripts. No 870 or 720 bp CD45R-IV^+ transcripts were observed.

mRNA transcripts (370 bp CD45R-0 product; Fig. 7 *B*). Furthermore, the blood and lung T cells contained similar levels of the CD45R-0^+ transcripts (blood 13 ± 2 density units, lung 14 ± 2 density units; $p > 0.7$; Fig. 7 *B*, *right*). As further evidence of the nonoverlapping expression of specific CD45 mRNA transcripts by lung $UCHL1^+$ T cells, single cell analysis of normal lung T cells demonstrated that 23 of 26 lung T cells expressed the CD45R-0^+ type of transcripts but none expressed CD45R-IV^+ transcripts (data not shown).

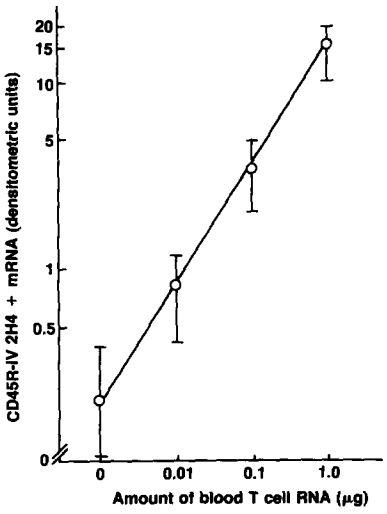

FIGURE 6. Quantification of CD45R-IV mRNA using cDNA synthesis, PCR amplification, Southern analysis with "probe IV," and laser densitometry. Data are shown as densitometry units. Ordinate: CD45R-IV mRNA levels (density units). Abscissa: blood T cell RNA (CD45R-IV$^+$) concentrations (μg/20 μl of cDNA synthesis medium) added to a fixed amount (1 μg/20 μl) of lung T cell RNA.

Cell Surface and mRNA Analysis of CD45 Expression by Blood and Lung T-cells in Chronic Beryllium Disease. Evaluation of the blood and lung T cells in individuals with chronic beryllium disease for the expression of CD45 isoforms using flow cytometry with the antibodies 2H4 and UCHL1 demonstrated that, similar to the normals, the lung T cell population was dominated by UCHL1$^+$ "memory" T cells. While blood T cells from these individuals comprised proportions of "naive" 2H4$^+$ and "memory" UCHL1$^+$ T cells that were similar to those observed in normals (2H4$^+$ 55 ± 4% of CD3$^+$, $p > 0.5$ compared with normal; UCHL1$^+$ 40 ± 4%, $p > 0.05$ compared with 2H4$^+$, $p > 0.1$ compared with normal), the lung T cells were dominated by UCHL1$^+$ "memory" T-cells (95 ± 2% of CD3$^+$; 2H4$^+$ 3 ± 1% of CD3$^+$, $p < 0.01$ UCHL1$^+$ compared with 2H4$^+$).

Consistent with these observations, PCR analysis of the levels of CD45 mRNA types in the T cells from the same individuals showed that T cells expressing the CD45R-0/UCHL1$^+$ mRNA accumulate in the lung of individuals with chronic beryllium disease. The lung T cells contained significantly less CD45R-IV mRNA than blood T cells (blood 35 ± 4 densitometric units [1.2 ± 0.3 log transformed units]; lung 2 ± 1 densitometric units [0.4 ± 0.3 log transformed units]; $p < 0.01$; Fig. 8], demonstrating that only a small proportion of lung T cells expressed the CD45 gene product in its "naive" nonspliced form. In contrast, lung T cells expressed significant levels of the CD45R-0 mRNA coding for the 180 kD UCHL1$^+$ protein [blood 20 ± 4 densitometric units (1.3 ± 0.1 log transformed units); lung 16 ± 5 densitometric units (1.1 ± 0.1 log transformed units); $p > 0.2$; Fig. 8] indicating that these lung T cells express the CD45R-0/UCHL1$^+$ but not the CD45R-IV/2H4$^+$ mRNA, i.e., they express the "memory" T cell phenotype of the CD45 gene. Finally, quantification of the absolute numbers of T cells on the epithelial surface of the lungs of normals and individuals with chronic beryllium disease showed that in the lungs of these patients, chronic antigen-specific inflammation drives the accumulation of exaggerated numbers of "memory" T cells. In this regard, the concentration of CD3$^+$ T cells present in the epithelial lining fluid of the lung of individuals with chronic beryllium disease was markedly increased (ninefold) compared with normal

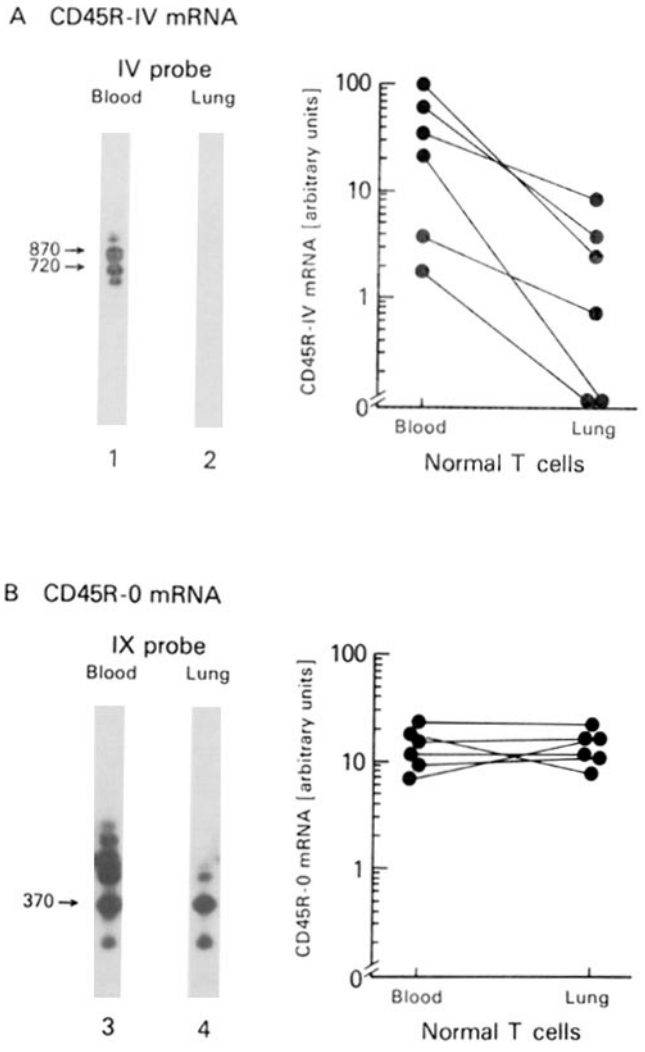

FIGURE 7. Evaluation of CD45 mRNA transcripts by normal blood and lung T cells. (*A*) Evaluation for CD45R-IV/2H4$^+$ mRNA transcripts. On the left is shown an example of the analysis using PCR and Southern analysis with a ^{32}P-labeled "IV probe." (Lane *1*) Blood T cell RNA. (Lane *2*) Lung T cell RNA. Arrows indicate the CD45R-IV$^+$ 870 and 720 bp products. On the right is shown the quantification of the relative amounts of CD45R-IV mRNA transcripts in blood and lung T cells of several normal individuals. The data are shown as arbitrary density units. (*B*) Evaluation for CD45R-0/UCHL1$^+$ mRNA transcripts. On the left is shown an example of the analysis using PCR and Southern analysis with a ^{32}P-labeled "IX probe." (Lane *3*) Blood T cell RNA. (Lane *4*) Lung T cell RNA. The arrow indicates the CD45R-0 370 bp product. On the right is shown the quantification of CD45R-0 mRNA transcripts in the blood and lung T cells of several normal individuals. The data are shown as arbitrary density units. In *A* and *B*, each data point represents a single individual and the lines indicates the data from the same individual.

(30.0 ± 14.0 × 10^3 cells/μl ELF [4.3 ± 0.2 log transformed concentration]; normal 3.2 ± 0.9 × 10^3 [3.3 ± 0.1 log transformed concentration]; $p < 0.01$; Fig. 9). Strikingly, within the lung CD3$^+$ T cell population, the total number of "naive" 2H4$^+$ T cells in chronic beryllium disease was similar to normal (CD3$^+$2H4$^+$, 0.9 ± 0.5 × 10^3 cells/μl ELF [2.6 ± 0.2 log transformed concentration]; normal 0.2 ± 0.2 × 10^3 [2.1 ± 0.2 log transformed concentration); $p > 0.1$; Fig. 9), but the number of "memory" UCHL1$^+$ T cells was markedly increased compared with normal (CD3$^+$UCHL1$^+$, 27.2 ± 12.1 × 10^3 cells/μl ELF [4.2 ± 0.1 log transformed concentration]; normal 2.9 ± 0.9 × 10^3 [3.2 ± 0.2 log transformed concentration]; $p < 0.01$; Fig. 9), and accounted entirely for the increase in the T cell numbers in the lower respiratory tract of these individuals. Thus, the T cell populations in an antigen-driven chronic inflammatory disorder have the same "memory" type char-

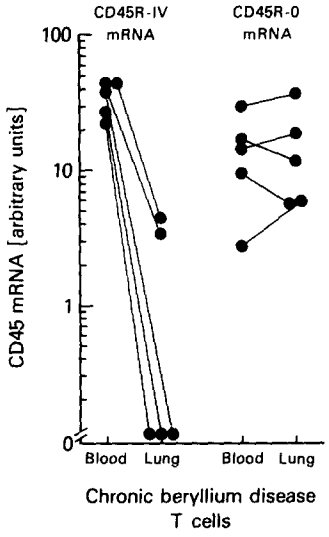

FIGURE 8. Evaluation of CD45 mRNA expression by blood and lung T cells of individuals with chronic beryllium disease. The analysis was identical to that for the normal individuals using the "IV probe" (CD45R-IV mRNA transcripts) and "IX probe" (CD45R-0 mRNA transcripts). Data are shown as arbitrary density units. Each data point represents a single individual. The lines connect the data from the same individual.

acteristics as it is found in the normal lung, but the inflammatory disease has a markedly increased total number of these cells.

Discussion

All T cells are characterized by their surface expression of the α/β or γ/δ antigen receptors (40). The role of the γ/δ receptor is not fully defined (41), but the TCR-α/β clearly imparts upon T cells the ability to bind to antigens in the context of major histocompatibility molecules of class I (cytotoxic T cells) or class II (helper T cells) (42). While all T cells have the potential to bind to specific antigens (40, 42), it has become clear that T cells can be classified into two populations that respond to anti-

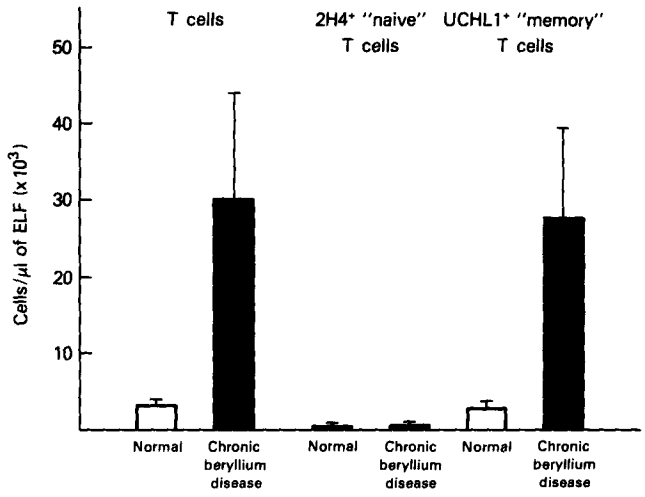

FIGURE 9. Quantification of the numbers of CD^+2H4^+ "naive" T cells and $CD3^+UCHL1^+$ "memory" T cells on the epithelial surface of the lower respiratory tract of normals and individuals with chronic beryllium disease. The analysis was by flow cytometry with the antibodies Leu4 (CD3), 2H4 ($CD45R\text{-}IV^+$), and UCHL1 ($CD45R\text{-}0^+$) as indicated in Fig. 2. Data are presented as absolute numbers of $CD3^+$, $CD3^+$ $2H4^+$, and $CD3^+UCHL1^+$ T cells/μl of ELF recovered.

gens differently (1–4, 43). "Naive" T cells do not respond well to recall antigens (1–4), but do proliferate to some extent in response to anti-CD3ε chain antibodies in the presence of IL-2 (44) or factors released by $CD4^+$ T-cells (45). In contrast, "memory" T cells of either the $CD4^+$ and the $CD8^+$ subsets respond to recall antigens (46, 47), proliferate strongly following CD3ε stimulation (43), and do not require exogenous IL-2 (43–45), characteristics of T cells competent to initiate immune responses to recall antigens. Current concepts hold that postthymic T cells with the "naive" T cells phenotype undergo further differentiation to become "memory" T cells, a process that entails enhanced expression of a number of adhesion molecules such as CD2, LFA1, and CDW29, the loss of surface $CD45/2H4^+$ proteins, and the acquisition of the expression of $CD45/UCHL1^+$ proteins (1–5, 48, 49). In the context that tissues such as the epithelial surface of the lung are chronically exposed to a variety of antigens, the present study was directed toward defining the status of the T cell populations on the pulmonary epithelial surface in regards to their state of differentiation as "naive" or "memory" T cells, i.e., whether the normal tissue T cell population at a site chronically exposed to antigens is specialized in responding to recall antigens, or is as heterogeneous as are blood T cells.

Interestingly, we found that the population of T cells in the normal lung are dominated by $CD45R\text{-}0^+$, $UCHL1^+$ "memory" T cells. Furthermore, comparison of T cell high density expression of CD45 $2H4^+$ and $UCHL1^+$ proteins ("bright" $2H4^+$ and $UCHL1^+$ cells) with the expression of specific CD45 mRNA transcripts showed that, like the two populations of T cells in the blood, these two populations in the lung express alternative, nonoverlapping gene programs, i.e., $2H4^+$ T cells do not express the CD45R-0 mRNA and vice versa. The observations with the normal lung T cells suggest that the T cells that compartmentalize to and dominate the T cell populations on the lung epithelial surface are already differentiated, "memory" T cells. Several lines of evidence support this concept:

(*a*) Combined flow cytometry and mRNA analysis demonstrated that most lung $2H4^-UCHL1^+$ T cells expressed only the spliced CD45R-0 mRNA form, suggesting that the regulatory mechanism that directs tissue-specific splicing of CD45 RNA (50) had occurred previously. Consistent with this concept, single cell CD45 mRNA analysis of lung T cells showed they expressed CD45R-0 and not CD45R-IV mRNA transcripts.

(*b*) In vitro studies with blood T cells demonstrated that activation of "naive" T cells with appropriate stimuli induces progressive loss of surface expression of $2H4^+CD45$ and at the same time induces the expression of $UCHL1^+CD45$ (48, 49; and Sanders, M., Clinical Pharmacology, Upjohn Co., Kalamazoo, MI, personal communication).

(*c*) It is unlikely that lung T cell activation of the IL-2 gene and T cell proliferation drive normal lung T cells to switch from $2H4^+$ to $UCHL1^+$ in situ in the lower respiratory tract, since normal lung T cells are proliferating at very low rates (51) and are not expressing IL-2 mRNA (51, 52). In addition, consistent with the concept that lung T cells are already differentiated, lung T cells express the very late activation surface antigen VLA1 in much larger proportions than early activation surface proteins such as the IL-2 receptor (53).

(*d*) "Memory" T cells are likely to migrate into the normal lung at a higher rate than "naive" T cells. In this regard, as a consequence of a higher surface density

of CD2 and LFA-1 (3, 5, 19), adhesion molecules that bind to the LFA3 and ICAM-1 intercellular adhesion ligands (54), "memory" T cells have the increased ability of adhering to capillary endothelial cells and of negotiating tissues. Furthermore, because normal alveolar macrophages are capable of high levels of production of TNF-α (55), a potent cytokine capable of upregulating LFA3 and ICAM1 expression in a number of cells (56), lung exposure to airborne particles and antigens may contribute to activate the recruitment of significant numbers of differentiated "memory" T cells to the lung.

Together, these observations are relevant to the understanding of the normal immune response in the lung, in that they suggest that the lung is a "specialized" site of T cell response to recall antigens. These "memory" T cells have the potential of a prompt release of the macrophage-activating lymphokine IFN-γ, and a prompt, significant response to T cell receptor stimulation by recall antigens (5, 43). In the same context, the data are consistent with the concept that the lung epithelial surface may not be the site of primary immune responses. Although lower respiratory tract immunization can generate cytotoxic and IFN-γ-mediated recall immune responses (57, 58), this may be secondary to the transport of antigen to lung lymph nodes by macrophages (59) rather than local T cell stimulation. Furthermore, experiments with local lung Bacillus Calmette-Guerin vaccinations have shown that local lung immunization may be less efficacious than other vaccination routes (60), suggesting that T cell activation within the lymph nodes (a tissue that is rich in $2H4^+$ T-cells [61, 62]) may be necessary for optimal immunization against new antigens.

Further support of the concept that "memory" T cells compartmentalize to antigen-exposed tissues is provided by the observation that the chronic presence of a beryllium burden in the lower respiratory tract of individuals with chronic beryllium disease dramatically expands the numbers of $UCHL1^+$ T cells in the lung, i.e., the beryllium-specific $UCHL1^+$ T cells, already present or recruited to the lungs, are stimulated in the local milieu to proliferate. These observations are consistent with studies with anti-CD45 and CDw29 antibodies, demonstrating that at sites of chronic inflammation such as leprosy skin lesions (63), tuberculosis pleural effusions (16), and rheumatoid joint fluid (15, 17–19), T cells are dominated by $2H4^-/UCHL1^+/4B4^+$ ($CDw29^+$) T cells. This suggests that chronic exposure to antigens is characterized by the compartmentalization of larger numbers of "memory" type T cells in tissues (19). The observations in chronic beryllium disease also support the concept that immune response of this disease is a normal, albeit an exaggerated response, i.e., the exaggerated immune process results from a normal, although heightened response of compartmentalized antigen-specific "memory" T cells.

In conclusion, this study demonstrates that in the lung, a model of normal tissue chronically exposed to antigens, the local T cell population is dominated by "memory" $2H4^-$ $UCHL1^+$ T cells. The observation is important to the understanding of tissue-lung immunity in two ways. First, it puts the concept of postthymic differentiation of "memory" T cells (1–5, 48, 49) in the context of the role of T cell populations in tissue immune responses (64, 65). Second, by supporting and further defining the concept of the lung as an immune compartment, it is suggested that strategies for the evaluation of the immunoregulatory processes in chronic inflammatory lung disorders must be centered on evaluating "local" processes since evaluation of the

blood will not yield a valid picture of the immune processes ongoing within the tissue. Finally, the finding that the normal lung is the site of a specialized population of T cells has important implications to chronic immune disorders of the lung. In this regard, in the context of a number of studies demonstrating that the $2H4^+$ "naive" T cell population, but not the population of $UCLH1^+$ "memory" T cells, contains T cells capable of inducing suppressor T cells (30), it is possible that persistent activation of sensitized helper T cells in the lung milieu (such as in individuals with chronic beryllium disease) may not be accompanied by local generation of a down regulatory T cell response, with the concomitant suppressor T cell activation (23–25, 66).

Summary

Expression of alternatively spliced products of the CD45 leukocyte common antigen gene identifies two populations of blood T cells: "naive" T cells (containing CD45R-IV mRNA transcripts, CD45 220, 205 kD surface proteins detected with antibody 2H4) that respond poorly to recall antigens, and "memory" T cells (containing CD45R-0 mRNA transcripts, expressing CD45 180 kD protein, detected with antibody UCHL1) that respond promptly to recall antigens. While blood contains approximately equal numbers of "naive" and "memory" T cells, it is known that $UCHL1^+$ "memory" T cells accumulate at sites of chronic inflammation. To test the concept that "memory" T cells are a feature of the T lymphocyte populations present in tissues chronically exposed to antigens in normals as well as in individuals with chronic inflammation, we evaluated T lymphocytes obtained from blood and the epithelial surface of the lower respiratory tract of normal individuals for the expression of specific CD45 surface protein isoforms and corresponding mRNA transcripts. Flow cytometric analysis of CD45 220, 205, and 180 kD surface proteins demonstrated that lung T cells of normals are dominated by $UCHL1^+$ "memory" cells (86 ± 2%) while autologous blood T cells have equal proportions of "memory" $UCHL1^+$ and "naive" $2H4^+$ T cells. In addition, polymerase chain reaction analysis of CD45 mRNA transcripts revealed that the lung cells expressed CD45R-0 mRNA transcripts but 17-fold fewer CD45R-IV mRNA transcripts than autologous blood T cells ($p < 0.01$). The pattern of lung T cells being dominated by CD45R-0 $mRNA^+$, $UCHL1^+$ "memory" T cells was also observed in individuals with chronic beryllium disease, an example of a chronic inflammatory disease in which antigen-specific T cells accumulate on the pulmonary epithelial surface. Like the normals, the lung T cells of the beryllium disease patients were dominated by CD45R-0 mRNA $transcript^+$, $UCHL1^+$, T cells. However, on a quantitative basis, the beryllium patients contained far greater numbers of T cells, i.e., the T cell populations on the surface of the normal and inflamed lung are similar in character ("memory" T cells) but differ in numbers (there are far more in the chronic inflammatory state). Thus, T cell populations on the epithelial surface of the normal lung likely reflect the chronic exposure to a diverse set of antigens, with a pattern that is qualitatively similar to that observed among T cells accumulating in response to a single antigen.

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