Multimodally profiling memory T cells from a tuberculosis cohort identifies cell state associations with demographics, environment and disease

Aparna Nathan1,2,3,4,5, Jessica I. Beynor1,2,3,4,5, Yuriy Baglaenko1,2,3,4,5, Sara Suliman2, Kazuyoshi Ishigaki1,2,3,4,5, Samira Asgar1,2,3,4,5, Chuan-Chin Huang6,7, Yang Luo1,2,3,4,5, Zibiao Zhang6,7, Kattya Lopez2,8, Cecilia S. Lindestam Arlehamn9, Joel D. Ernst10, Judith Jimenez8, Roger I. Calderón8,11, Leonid Lecca6,8, Ildiko Van Rhijn2,12, D. Branch Moody2, Megan B. Murray6,7 and Soumya Raychaudhuri1,2,3,4,5,13

Multimodal T cell profiling can enable more precise characterization of elusive cell states underlying disease. Here, we integrated single-cell RNA and surface protein data from 500,089 memory T cells to define 31 cell states from 259 individuals in a Peruvian tuberculosis (TB) progression cohort. At immune steady state >4 years after infection and disease resolution, we found that, after accounting for significant effects of age, sex, season and genetic ancestry on T cell composition, a polyfunctional type 17 helper T (T(Th17)) cell-like effector state was reduced in abundance and function in individuals who previously progressed from *Mycobacterium tuberculosis* (*M.tb*) infection to active TB disease. These cells are capable of responding to *M.tb* pepti-ides. Deconvoluting this state—uniquely identifiable with multimodal analysis—from public data demonstrated that its depletion may precede and persist beyond active disease. Our study demonstrates the power of integrative multimodal single-cell profiling to define cell states relevant to disease and other traits.

T cells occupy a complex landscape of functional states characterized by combinations of mRNA, surface proteins, transcription factors and cytokines. These modalities individually lend limited insight into cellular function, but jointly they recapitulate the breadth of T cell states. However, profiling strategies frequently assay a single modality, average across heterogeneous states (bulk RNA sequencing (RNA-seq)), have limited detection efficiency for key markers (single-cell RNA-seq) or only target pre-defined phenotypes of interest (flow cytometry and mass cytometry). Recent technologies incorporate oligonucleotide-labeled antibodies into droplet-based single-cell sequencing to simultaneously measure surface markers alongside intracellular mRNA transcripts, enabling comprehensive characterization of T cell states. Interindividual differences in T cell state abundance and function can correlate with demographics (for example, age and sex) or environment. Moreover, they may underlie divergent disease outcomes, such as response to pathogens like *M.tb*—a leading infectious cause of death. Nearly a quarter of the world’s population is estimated to be infected with *M.tb*, but only 5–15% of infected individuals develop TB disease, causing an estimated 1.5 million annual deaths. Given the prevalence of infection and mortality upon progression to active disease, there is an urgent need to understand the immune correlates of progression risk. For example, people who previously progressed to active TB tend to have higher progression risk than others, which may indicate that persistent baseline immune differences reduce the capacity to control *M.tb* infection.

Previous studies implicate key memory T cell states in TB disease progression. However, T cell immunoprofiling studies often have key limitations: (1) insufficient clinical and demographic data to mitigate confounding factors and (2) profiling donors during disease, when disease-induced inflammation cannot be disentangled from inherent immune differences. Similarly, studies limited to antigen-specific cells may miss broader immune context. Here, we use cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) to profile >500,000 memory T cells from a TB progression cohort at post-disease immune steady state (that is, after treatment and TB disease resolution) and multimodally define cell
states associated with demographics, the environment and TB progression. We utilize this powerful resource to identify a T cell state associated with TB progression that responds to ex vivo *M. tb* peptide stimulation.

**Results**

**CITE-seq produces >500,000 multimodal T cell profiles.** We re-recruited 264 individuals from a larger epidemiological study (*n* = 18,544) in Lima, Peru that identified individuals with active TB disease and, for 1 year, followed their household contacts who were infected with *M. tb* to monitor progression to active disease (Fig. 1a)\(^1\). We re-recruited donors 4.72–6.60 years after initial recruitment and classified those who had originally been diagnosed with TB as cases. By the time of sample collection, cases had been treated for TB, which has an estimated cure rate of >95%, so we expected they had returned to immune steady state without disease-driven perturbation\(^19\). This facilitates discovery of states associated with disease outcomes, not merely activation during disease. Household contacts who had originally been positive for the tuberculin skin test (TST) and didn't develop TB disease by the time of re-recruitment were classified as controls. The cohort has comprehensive documentation of environmental (for example, socioeconomic status (SES), nutrition and smoking) and demographic (for example, age, sex and weight) traits. Consistent with the larger epidemiological study\(^18\), TB progression was associated with host traits such as age, height, weight, sex and body mass index (BMI; Supplementary Table 1).

We used magnetic sorting to negatively select CD45RA– memory T cells from peripheral blood mononuclear cells (PBMCs) from 131 cases and 133 controls (~98.4% purity; Fig. 1a). Using CITE-seq, we profiled single-cell RNA alongside an optimized panel of 31 oligonucleotide-tagged antibodies for surface proteins (Supplementary Table 2), including markers of lineage (for example, CD4 and CD8), activation (CD25 and HLA-DR), migration (CCR6 and CXCR3) and mouse immunoglobulin G as a control\(^4\). Subsequent cell-level and sample-level quality control (QC; Fig. 1b,c and Extended Data Fig. 1d), the final dataset contained 500,089 memory T cells from 259 individuals (mean, 1,845 cells/sample; 95% confidence interval (CI), 518–3,172; Fig. 1d). By sorting equal numbers of cells where possible, we observed no significant difference in cell count per sample between progressors and non-progressors (two-sided *t*-test *P* = 0.33; Extended Data Fig. 1d).

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**Fig. 1 | Study design and quality control.** a. We obtained PBMCs from a Peruvian TB cohort (*n* = 264 donors and 12 technical replicates, over 46 independent experiments), profiled memory T cells with CITE-seq and integrated multimodal single-cell profiles to define cell states and case–control differences. b. Cell counts over six QC steps. c. Single-cell QC metrics. Each cell was plotted according to the proportion of MT UMIs and the number of genes expressed. QC thresholds are demarcated with dashed lines. Counts indicate the number of cells in each quadrant. d. Distribution of post-QC cell yields for 259 samples. e. Schematic of CCA.
Multimodal integration defines 31 memory T cell states. As suggested by previous T cell phenotyping efforts, we assume that biologically relevant states have both mRNA and surface protein signatures. To integratively define high-resolution memory T cell states, we used canonical correlation analysis (CCA) to project each cell into a low-dimensional space defined by correlated modules of mRNA transcripts and proteins (Fig. 1e). This leverages modality-specific signatures; for example, regulatory T (Treg) cells have high surface expression of CD25 and absence of CD127 but also express FOXP3 transcripts, so we expect to find one or more dimensions driven by correlated variation in CD25, CD127 and FOXP3 that delineate Treg cells. We selected the top 20 canonical variates (CVs) with the highest mRNA–protein correlations (Extended Data Fig. 3a,b). The first CV, capturing the most shared variation, correlated with a previously defined gene expression signature of effector potential (Pearson r = 0.90; Extended Data Fig. 3c,d).28

Batch correction and graph-based clustering of the top 20 CVs defined 31 putative cell states with gene and protein markers (Fig. 2; Extended Data Fig. 4 and Supplementary Table 4).29 Based on surface protein, the majority (23/31) were CD4+ five were CD8− and one (C-24) was a mixture. Two clusters (C-30 and C-31) were CD4−CD8+, likely containing mostly γδ T cells that expressed TRDC, the constant region of the T cell antigen receptor (TCR) delta chain, but not the αβ TCR surface protein. Despite limited resolution of unconventional T cells with 3′ mRNA and these surface markers, one cluster (C-20) contained a subset of CD4+CD8− and CD8+ cells expressing innate-like T cell markers, including ZBTB16 and CD161 and CD26 surface proteins.31,32

Some CD4+ clusters resembled known T cell phenotypes, such as central memory cells (CD62L+33–35) and Treg cells (C-5 and C-9, CD25+CD127− and expressing FOXP3)36,37. Among CD8+ clusters, we identified one central memory cluster (C-25) and distinct GZMK+ (C-28) and GZMB+ (C-29) effector subsets, reflecting different cytotoxicities.38 Clusters with high expression of HLA-DR and CD38 surface protein and proliferation-associated MKI67 (C-15 and C-27) represent chronically activated cells.39

In addition to Treg cells (C-12, CCR6+ and RORC+) and type 1 helper T cells (T(H1; C-17, CXC3+ and IFNG and TBX21))40,41, we identified a heterogeneous continuum of intermediate T(H1/T(H17) states (C-13, C-16 and C-19) with varying degrees of CXCR3, CCR6, CCR5 and CD161 surface protein expression and RORC and TBX21 expression.11

We also identified disease-associated subsets of broader states. For example, we identified a CD161+ subset of type 2 helper T (T(H2)) cells (C-14), previously described as pathogenic, with higher expression of allergy-associated HPGDS and IL17RB.42 We found a subset of FOXP3+ Treg cells (C-5) expressing higher CCR6 surface protein and CTLA4 and RORC than other Treg cells, resembling Treg cells found in tumors and autoimmune diseases.33,34. As previous studies in blood have shown, CCR6+ memory Treg cells have higher CD161 surface protein and lower TIGIT surface protein and IKZF2 expression. We also found reduced HLA class II mRNA and surface protein expression, as well as decreased expression of RBTN2, CCR10 and TNFRSF9 and higher CD38 surface protein compared to other memory Treg cells (C-9). These subsets have not been well defined previously due to limited ability to align definitions between assays.

Memory T cell states vary with demographics and environment. Cell state abundance varied across donors but was correlated between technical replicates (Pearson r = 0.44–1.00; Fig. 3a and Extended Data Fig. 5), indicating that T cell states may be associated with other donor traits. We tested 38 demographic, socioeconomic and genetic ancestry covariates (Supplementary Table 5) for association with T cell states with mixed-effects modeling of associations of single cells (MASC)43, a single-cell cluster association model that accounts for confounders at the cell and donor levels. We corrected for donor, batch and total unique molecular identifiers (UMIs) and percentage of mitochondrial (MT) UMIs per cell, which all influence a cell’s cluster membership.

For each covariate, we quantified its association with memory T cell composition by aggregating independent cluster association P values into a gamma-distributed test statistic (Methods and Supplementary Fig. 2). Age, sex, winter blood draw and proportion of European genetic ancestry were significantly and independently associated with T cell state composition (Fig. 3b–e). As previous studies suggest,44 age had the strongest influence (gamma P = 2.24 × 10−15), associated with 12/31 states (univariate likelihood-ratio test (LRT) P < 1.6 × 10−5; 0.05/31). Similarly to prior findings,45,46, cytotoxic CD4+ T cells (C-23) were expanded by ~20% per decade of age (odds ratio (OR), 1.19; 95% CI, 1.10–1.28; P = 1.59 × 10−5). We also observed that CD8+ T cells (C-31) were reduced by >50% per decade (OR, 0.46; 95% CI, 0.39–0.54; P = 5.72 × 10−3; Fig. 3b and Supplementary Table 5).

Sex was also strongly associated with T cell states (gamma P = 8.40 × 10−8), and we further dissected published trends, such as a lower CD4:CD8 ratio in males and the inhibitory effect of male hormones on T(H1) differentiation.47 We observed expansion of GZMB+CD8+ T cells in males (C-29: male versus female OR, 1.88; 95% CI, 1.46–2.42; P = 1.72 × 10−4) and T(H1) cells in females (for example, C-17: male versus female OR, 0.77; 95% CI, 0.71–0.83; P = 1.20 × 10−11; Fig. 3c and Supplementary Table 5).

We observed surprising expansion of T(H1)2 states in samples collected in winter (gamma P = 4.40 × 10−4; for example, C-11; OR, 1.24; 95% CI, 1.10–1.39; P = 5.13 × 10−4; Fig. 3d and Supplementary Table 5). To our knowledge, this has not been reported previously, beyond general seasonality of cytokine responses.48

Immune function is thought to vary with genetic ancestry49, and in our cohort, all three cytotoxic CD4+ clusters were nominally depleted in individuals with higher European genetic ancestry (gamma P = 2.21 × 10−3, for example, C-23: OR, 0.14 per 100% European genetic ancestry; 95% CI, 0.05–0.48; P = 8.70 × 10−4; Fig. 3e and Supplementary Table 5).

These associations were significant independently of each other and TB progression status (Extended Data Fig. 6 and Supplementary Tables 6 and 7). Other covariates did not have significant associations with memory T cell composition after adjusting for age, sex, season and genetic ancestry (Fig. 3f).

An RORC+ effector state is reduced in TB progressors. Next, we sought to identify memory T cell states associated with M.tb infection outcome. We used MASC to test associations between each cell state and TB disease progression, adjusting for potentially confounding covariates (age, sex, winter blood draw and proportion of European genetic ancestry) and batch and single-cell technical factors (Methods).

We observed a significant 20% reduction in cluster C-12 in individuals who had progressed to active TB disease (OR, 0.80; 95% CI, 0.73–0.87; P = 1.21 × 10−4 < 0.05/31; Fig. 4a and Supplementary Table 7). Notably, C-12 was independently reduced with age (OR, 0.82; P = 2.69 × 10−3) and in males (OR, 0.85; P = 4.30 × 10−4) and expanded in winter (OR, 1.16; P = 1.30 × 10−2; Fig. 4b). This covariate-aware, multimodal strategy detects even a modest case–control difference in C-12 frequency (mean, 3.0% in cases and 3.6% in controls; Fig. 4c).
**Fig. 2 | Landscape of memory T cell states.**

**a.** Uniform manifold approximation and projection (UMAP) colored by 31 multimodal clusters. Cluster annotations were based on the top differentially expressed genes and surface proteins. Clusters boxed in red are CD4+ T_{eff}, purple are mixed CD4+ and CD8+, blue are CD8+ and green are CD4−CD8−. **b.** Expression of major lineage-defining surface proteins measured through CITE-seq. Colors are scaled independently for each marker from minimum (blue) to maximum (yellow) expression. **c.** Heat map of selected marker genes. Surface protein heat map colors are uniformly scaled for each protein. mRNA heat map colors reflect z-scores for each gene.
Fig. 3 | Memory T cell state associations with demographic and environmental factors. a, Distribution of cluster proportions across donors (n = 259). Box plots show the median (vertical bar), 25th and 75th percentiles (lower and upper bounds of box, respectively) and 1.5 times the interquartile range (IQR; or minimum/maximum values if they fall within that range; end of whiskers). Only nonzero proportions are plotted. b–e, Effects of age, sex, winter blood draw and proportion of European genetic ancestry in univariate model with correction for technical covariates (number of UMIs per cell and percentage of MT UMIs per cell), donor and batch. Error bars show the 95% CI. f, Associations of covariates with T cell composition. Each column represents associations from a MASC model fit with the indicated covariate (row) as the contrast, and correction for the indicated covariates (cumulative column headings, from left) as fixed effects and donor and batch as random effects. Heat map colors correspond to gamma test P values; white indicates that the covariate is not significant (NS) after multiple-testing correction (P > 0.05/38), and gray indicates that the covariate has already been added to the model. Age and age squared are linear and quadratic terms of age at blood draw. Technical effects are the number of UMIs per cell and the percentage of MT UMIs per cell. IPT, isoniazid preventative therapy. Data are from n = 271 samples from 259 independent donors.
Cells in C-12 have a CD4+ effector surface phenotype (CD62L: expression fold change C-12 versus other (FC) = 0.66, \( P = 2.99 \times 10^{-7} \); CCR7: FC = 0.85, \( P = 9.72 \times 10^{-3} \)) and lack surface markers of activation (HLA-DR: FC = 0.39, \( P = 1.86 \times 10^{-3} \)) or exhaustion (PD-1: FC = 0.76, \( P = 7.23 \times 10^{-4} \)). The top surface proteins were CD26, CCR6 and CD161, and the top transcripts were CCR6, CTSH and KLRB1 (Supplementary Table 4), with elevated expression of T\(_{\text{R1}}\) lineage-defining transcription factor RORC (FC = 5.70, \( P = 2.37 \times 10^{-10} \)) compared to all other memory T cells. We also noted reduced expression of T\(_{\text{R1}}\) lineage-defining TBX21 (FC = 0.52, \( P = 1.01 \times 10^{-4} \)) and IFNG (FC = 0.30, \( P = 7.23 \times 10^{-3} \)), although absence of transcripts encoding transcription factors or cytokines may reflect poor detection or a paucity of preformed mRNA\(^2\). There were no differentially expressed genes between cases and controls in this cluster. This combination of markers suggests that C-12 is a T\(_{\text{R1}}\)17 subset\(^2\).

When clustering on single modalities—either mRNA or protein—we were unable to precisely capture C-12 (Extended Data Fig. 7a–f). Accordingly, no mRNA-based clusters were significantly associated with TB progression (Fig. 4d and Supplementary Table 8). Two protein-based clusters partially overlapping C-12 were reduced in cases, but less significantly than in controls (Extended Data Fig. 7g and Supplementary Table 8). Another multimodal cluster (C-20, innate-like T cells) was also reduced in cases but with modest significance (mean, 1.1% in cases and 1.2% in controls; OR, 0.76; 95% CI, 0.65–0.89; \( P = 5.95 \times 10^{-4} \); Extended Data Fig. 7h).

Because our cohort was profiled years after TB diagnosis and treatment, we expected minimal differences in activation states. Using MASC, we found no differences in CD4\(^{+}\)HLA-DR\(^{+}\) memory T cells (OR, 1.02; 95% CI, 0.86–1.22; \( P = 0.79 \)). Additionally, because we profiled all memory T cells, progression-associated differences identified in previous studies of M.\(\
\text{tb}\) antigen-specific T cells were only marginally significant (Supplementary Table 9).

**Latency and treatment are unlikely to drive C-12 association.** To assess whether reduced C-12 abundance is a steady-state correlate of progression risk, we considered the alternatives that C-12 is increased by latent TB or decreased by active TB. We defined a gene expression score based on sorted T cells and bulk PBMCs that estimates C-12 frequency with high cross-validation accuracy (T cell Pearson \( r = 0.78 \); PBMCs \( r = 0.74 \); Extended Data Fig. 8a) and used it to estimate the C-12 proportion in public bulk expression data from studies of latent infection or disease (Methods). The highest-weighted genes were C-12 markers, including CCR6 and KLRB1 (Supplementary Data). As a negative control, we defined a score for C-11 (T\(_{\text{R2}}\), cross-validation \( r = 0.73 \)), which was not associated with disease progression.

We applied the C-12 score to a cohort from the United Kingdom including healthy (uninfected) donors, latently infected donors and M.\(\
\text{tb}\)-infected adolescents \( \geq 1 \) year before disease progression in cases (avoiding effects of preclinical disease) or controls with continued latent infection \( (n = 54 \text{ donors at } 1–2 \text{ time points}; \text{Extended Data Fig. 8f}) \). Using a mixed-effects linear model correcting for age, sex, sequencing platform and donor, we found that predicted C-12 abundance was 9% lower in cases before progression compared to latent controls \( (\Delta \text{frequency}_{\text{case-control}} = -0.0031, \text{one-sided } t\text{-test of } \beta_{\text{case-control}} \text{ P} = 0.038) \). This effect is comparable to the 13% decrease in C-12 proportion computed with the same predictor in former progressors at post-disease steady state in the Peruvian CITE-seq (LIMAA) cohort \( (\Delta \text{frequency}_{\text{case-control}} = -0.0047; \text{Fig. 4e and Extended Data Fig. 8g}) \). Thus, differences in C-12 abundance likely precede disease progression.

We found no difference in the C-11 score before disease progression \( (\Delta \text{frequency}_{\text{case-control}} = -0.0011, \text{one-sided } t\text{-test of } \beta_{\text{case-control}} \text{ P} = 0.23; \text{Fig. 4c}) \), demonstrating that the reduction is not in all T cell states. We also assessed whether the C-12 score was spuriously correlated with other clusters and observed that it was most likely predate disease progression.

**Public data show C-12 depletion may precede TB progression.** Next, we considered whether the C-12 reduction seen at post-disease steady state is an antecedent or consequence of disease. Using the C-12 score trained on T cells and PBMCs, we estimated C-12 abundance in bulk RNA-seq of sorted T cells from a South African cohort of M.\(\
\text{tb}\)-infected adolescents \( \geq 1 \) year before disease progression in cases (avoiding effects of preclinical disease) or controls with continued latent infection \( (n = 54 \text{ donors at } 1–2 \text{ time points}; \text{Extended Data Fig. 8f}) \). Using a mixed-effects linear model correcting for age, sex, sequencing platform and donor, we found that predicted C-12 abundance was 9% lower in cases before progression compared to latent controls \( (\Delta \text{frequency}_{\text{case-control}} = -0.0031, \text{one-sided } t\text{-test of } \beta_{\text{case-control}} \text{ P} = 0.038) \). This effect is comparable to the 13% decrease in C-12 proportion computed with the same predictor in former progressors at post-disease steady state in the Peruvian CITE-seq (LIMAA) cohort \( (\Delta \text{frequency}_{\text{case-control}} = -0.0047; \text{Fig. 4e and Extended Data Fig. 8g}) \). Thus, differences in C-12 abundance likely precede disease progression.

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**a**

Graph showing the abundance per donor of a specific marker (C-12) in case vs. control groups. The x-axis represents OR case vs. control, and the y-axis represents the negative logarithm of the p-value. Different markers (C-12, C-20, C-16, C-19, C-17, C-23, C-29, C-4, C-24, C-28, C-22) are plotted with their corresponding p-values.

**b**

Table listing the variables and their associated MASC P-values:

- TB progression (case vs. ctrl): $1.21 \times 10^{-6}$
- Age (per 10 years): $2.69 \times 10^{-3}$
- Sex (M vs. F): $4.30 \times 10^{-4}$
- Season of blood draw (winter vs. other): $1.30 \times 10^{-3}$
- Prop. EUR genetic ancestry (per 100%): $0.02$

**c**

Scatter plot showing the abundance per donor of marker C-12 in case (progressors) and control (non-progressors) groups. The y-axis represents the abundance per donor, and the x-axis represents the OR case vs. control.

**d**

Graph showing the difference in cell state proportion (case–control) for the marker M-24. The x-axis represents the OR case vs. control, and the y-axis represents the negative logarithm of the p-value.

**e**

Comparison of difference in cell state proportion (case–control) for markers C-12, C-11, and C-12 with pre-disease and post-disease steady state data. The p-values are $P = 0.038$, $P = 2.4 \times 10^{-5}$, and $P = 0.23$ respectively.
correlated with C-12 abundance. The score’s correlation with abundances of other clusters corresponds to the correlations of those clusters with true C-12 proportion (Extended Data Fig. 8h). For example, the proportion of C-23 (cytotoxic CD4+CD8−) was negatively correlated with both C-12 proportion and score, while the proportion of C-9 (T reg cells) had approximately a correlation of 0 with C-12 proportion or score.

CD4+CD26+CD161+CCR6+ captures a TB-associated T cell state. Functional characterization of C-12 required ex vivo sorting based on surface markers of the multimodally defined cluster. In addition to observed surface expression of TCRαβ and CD4, we built a classification tree and used stepwise selection to identify a minimal set of candidate markers: CD26+, CD161+ and CCR6+ (sensitivity of 54.8% and specificity of 95.5%; Fig. 5a–d and Methods). While these markers captured not just C-12 cells, C-12 comprised the plurality of cells and likely made the most significant functional contributions. The majority of non-C-12 cells in the gate came from the C-4 cluster, with high CD27 and CCR7 surface proteins and lacking RORC and TBX21 expression, suggesting less differentiation and effector potential.

As expected, MASC modeling demonstrated a reduction of this population in TB progressors when gated from CITE-seq data (OR = 0.75, P = 3.56 × 10−4; Fig. 5e and Supplementary Fig. 3a).
Removing any individual gate weakens the association. Therefore, these C12 surface markers define a disease-associated population that is a sortable proxy for ex vivo investigation.

**TB-associated state makes IL-17 and IL-22 upon stimulation.** To define the cytokine profile of C12, we isolated CD4 T cells from three Boston-based donors—likely M.tb infection-naive given the low TB burden there—and sorted CD45RO⁺CD26⁺CD161⁺CCR6⁺ cells (Supplementary Fig. 3b and Supplementary Table 10a). For comparison, we sorted naive CD4+ cells, other memory CD4+ cells and Treg cells (Methods).

We stimulated each population with CD3/CD28 beads for pan-TCR activation and measured helper T cell cytokines in the supernatant. Compared to other memory CD4+ T cells, the target population produced more IL-17A, IL-17F and IL-22 and less IL-4 and IL-13 (two-sided t-test; IL-17A: t = 5.07, \( P = 0.04 \); IL-17F: \( t = 6.34, \ P = 0.02 \); IL-22: \( t = 8.00, \ P = 0.012 \); IL-4: \( t = -6.96, \ P = 0.02 \); IL-13: \( t = -4.44, \ P = 0.02 \); Fig. 6a). To determine if this was robust
to stimulation condition and assay, we stimulated cells from five Boston donors with phorbol 12-myristate 13-acetate (PMA) and ionomycin. With intracellular staining, we again found that our target population was more likely to produce IL-17A, IL-17F and IL-22 than other CD4+ memory T cells (Cochran–Mantel–Haenszel (CMH) OR; IL-17A: 12.6; IL-17F: 18.1; IL-22: 6.0; all P < 0.001; Fig. 6b–d and Extended Data Fig. 9). Inverting any surface marker (CD26, CD161 and CCR6) reduced the proportion of cells expressing IL-17A, IL-17F and IL-22 (Fig. 6f and Extended Data Fig. 9). This indicated a T17-like phenotype. Furthermore, the target population was polyfunctional: although it produced interferon (IFN)-γ and TNF at rates similar to those of CD4+ memory T cells overall (CMH OR; IFN-γ: 0.72; TNF: 1.59 all P < 0.001), it had more than three times as many IFN-γ-producing cells as the rest of the T117 compartment (two-sided t-test versus CD26-CD161+CCR6+, P = 2.23 × 10−6; Fig. 6g).

**TB-associated state makes IL-17 at lower rates in progressors.** Because IL-17 and IL-22 best characterized this population’s functional phenotype in non-Peruvian donors, we next measured these cytokines in Peruvian donors with a history of *M. tb* infection. We selected eight pairs of cases and controls from our original CITE-seq cohort, matched for age, sex, season of blood draw and proportion of European ancestry. Per-donor percentage of all cells producing IL-17A (left) or IL-22 (right) in each gated population. In *d* and *e* we compared T cell response to *M. tb* in long-term immunity upon pathogen exposure. One practical disease-induced inflammation. Memory T cells are also involved in the landscape of progression-associated memory T cell differences outside disease-induced inflammation. Memory T cells are also involved in long-term immunity upon pathogen exposure. One practical limitation of our approach is excluding naive and CD45RA+ TEMRA cells. This large dataset captures even rare memory subsets, defined through multimodal single-cell data. Moreover, by profiling donors

**TB-associated state has IL-17/IFN-γ response to *M. tb* peptide.** We investigated whether C-12 contains cells that respond to *M. tb* antigens. We stimulated PBMCs from six additional Peruvian donors with prior *M. tb* infection ≥4 years earlier (three progressors and three non-progressors) with an *M. tb* peptide megapool (*MTB300*; Methods). Given its T17-like phenotype with IFN-γ production capacity, we measured IL-17A and IFN-γ in the target population defined by the surface markers of C-12 (CD4+CD45RO+CD26+CD161+CCR6+; Extended Data Fig. 10a,b). We assayed two Boston-based control donors who were likely to be naive to *M. tb*, given lack of IL-17A or IFN-γ T cell response to *M. tb* stimulation (Extended Data Fig. 10c). We observed a response to MTB300 in the C-12 cells from formerly infected Peruvian donors: an average of 1.05% of cells per donor in the target population produced IL-17A or IFN-γ with antigen stimulation, 2.3-fold higher than without antigen (Wilcoxon signed-rank *P* = 0.031; Fig. 7g). In comparison, only 0.26% of target cells in controls produced IL-17A or IFN-γ upon megapool stimulation (one-sided Wilcoxon rank-sum test (TB cohort versus control, with *M. tb* stimulation), *P* = 0.036).

**Discussion**

In immunoprofiling studies, T cells repeatedly emerge as a likely contributor to disease, but further investigation has been limited by the complexity of cell states and their interactions with non-immune factors. Here, we present an atlas of >500,000 memory T cells with unbiased multimodal single-cell measurements from a TB progression cohort with extensive demographic and environmental data. After defining cell states with a new integrative approach, we identified associations between state frequencies and demographic and environmental factors, particularly age, sex, and genetic ancestry. Understanding how these factors influence T cells can inform the design of more robust T cell profiling experiments, for example, by ensuring experimental groups are unbiased by these confounders or adjusting for them post hoc during analysis.

In this study, we leveraged the unique technical and analytical advances of this memory T cell dataset to identify states associated with TB disease progression. We focused on memory T cells because of previous studies that found immune evidence that TB progression is influenced by host memory T cells but that did not comprehensively illuminate the landscape of progression-associated memory T cell differences outside disease-induced inflammation. Memory T cells are also involved in long-term immunity upon pathogen exposure. One practical limitation of our approach is excluding naive and CD45RA+ TEMRA cells. This large dataset captures even rare memory subsets, defined through multimodal single-cell data. Moreover, by profiling donors

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**Fig. 7** Characterizing C-12 as an IL-17+ state with reduced function in Peruvian TB cases. a, Correlation between abundance of flow-gated population (CD4+CD45RO+CD26+CD161+CCR6+) and C-12, per donor. b, Correlation between abundance of flow-gated population (CD4+CD45RO+CD26+CD161+CCR6+) and the in silico–gated population, per donor. In a and b, we calculated a linear best-fit line and Pearson correlation coefficient (*r*) across 16 donors. c, Per-donor percentage of cells producing IL-17A (left) and IL-22 (right) in populations gated in Peruvian TB cohort donors. d, Per-donor percentage of all cells producing IL-17A (left) or IL-22 (right) in each gated population. In c and d, bars represent the mean, and error bars show the s.e.m. across 16 donors. e, IL-17A or IL-22 production in gated populations. Data are presented as CMH ORs of cytokine production inside versus outside the gate (95% CI error bars; *n* = 16 independent samples). f, Case-control comparison of the per-donor percentage of cells in indicated gates producing IL-17A (top) or IL-22 (bottom). Paired samples were matched for age, sex, season of blood draw and proportion of European ancestry. *P* values are from a one-sided Wilcoxon signed-rank test. g, Mtb-specific IL-17A or IFN-γ response to MTB300 stimulation in CD4+CD26+CD161+CCR6+ memory T cells from either Boston control donors (*n* = 2) or Peruvian TB cohort donors (*n* = 6). Each point corresponds to the percentage of cells producing IL-17A or IFN-γ from one donor, measured with intracellular cytokine staining. Lines connect measurements from the same donor before and after stimulation with MTB300 peptide megapool. *P* values are from a two-sided Wilcoxon signed-rank test comparing donors before and after antigen stimulation. In f and g, box plots show the median (vertical bar), 25th and 75th percentiles (lower and upper bounds of the box, respectively) and 1.5 times the IQR (or minimum/maximum values if they fall within that range; end of whiskers).
in the absence of active disease, this resource is suitable for further investigation of unperturbed memory T cell phenotypes.

We found that the most significant steady-state differences between progressors and non-progressors reside in a rare (3%) of memory T cells) multimodally defined T₈₁7 subset (C-12) marked by a CD₄⁺CD₄₅₆⁻CD₁₆₁⁺CCR₆⁺ surface phenotype. These markers have each previously been associated with the IL-17-producing T₈₁7 state and other functions: CD26 is a co-stimulatory molecule that promotes cytotoxicity, and CD161 is associated with innate-like function; and CCR6 is a homing marker that directs migration to inflamed sites.

We assayed donors who recovered from TB, suggesting that observed differences in the abundance and function of C-12 may either be a long-term consequence of prior TB disease or predispose individuals to TB disease progression. Previous immunoprofiling studies have shown that changes in the T cell compartment persist immediately after TB disease and wane with time. Although our study design limits our ability to conclusively disentangle disease...
sequelae from baseline differences, there is evidence suggesting that reduction in C-12 may precede disease.

First, we demonstrate in previously published cohorts that despite modulation during ongoing TB disease or treatment, the gene expression signature of C-12 is elevated in latently infected controls compared to active TB cases even 1 year before progression. We trained a linear model on memory T cell and PBMC data and used it to estimate C-12 frequencies in a large public dataset from a closely matched cell type (sorted T cells) assayed before disease, effectively establishing confidence in these inferences.

Second, mutations in RORC—the gene encoding the Th17 lineage-definition transcription factor that is highly expressed in C-12—increase susceptibility to mycobacterial diseases. Mutations in IL17A and IL17F have also been associated with TB susceptibility, and blocking IL-17 in mice reduces capacity for anti-M. tb immunity after vaccination.

Third, we show that C-12 cells respond to M. tb peptide antigens with IL-17A or IFN-γ production. Previous studies in latent infection have mapped the bulk of M. tb antigen-specific cells to a Th1i/Th17 CCR6+CXCR3+ state (distinct from C-12) that is expanded in latent infection compared to uninfected individuals. Although these cells produced only IFN-γ upon ex vivo M. tb antigen stimulation in those studies, in nonhuman primates, they also produce IL-17 in bronchoalveolar lavage and expand in the lungs during latent infection compared to TB disease and upon intravenous Bacillus Calmette-Guérin (BCG) vaccination. IL-17 and IL-22 production and other aspects of the C-12 phenotype have also been previously identified in M. tb antigen-specific cells during active TB disease. Considering previously described TB vaccine-induced Th1 polarization of lung-homing memory Th17 cells in mice, the polyfunctional response of C-12 to M. tb peptides may reflect anti-mycobacterial potential, and this state may be a worthwhile target in vaccine design.

The potential of C-12 to be a baseline correlate of TB progression risk is further supported by its depletion with increased age, in males and outside of winter, all of which have been associated with TB risk. These host variables may in part increase TB risk by reducing C-12 frequencies. Additionally, C-12 does not have an activated or exhausted phenotype, arguing that it is not the consequence of chronic stimulation. Further prospective studies profiling the immune system before infection are required to conclusively link baseline C-12 with response to M. tb infection.

Our results demonstrate the power of high-dimensional multimodal T cell profiling to identify steady-state immune differences between divergent disease outcomes. With consistent results across multiple modalities and datasets, we offer evidence supporting a hypothesis for how differences in memory T cell composition may reflect inadequate host response to M. tb. We also provide a resource to enable further robust study of numerous memory T cell phenotypes.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at [https://doi.org/10.1038/s41590-021-00933-1](https://doi.org/10.1038/s41590-021-00933-1).

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**References**

1. Nathan, A., Baglaenko, Y., Fonseka, C. Y., Beynor, J. I. & Raychaudhuri, S. Multimodal single-cell approaches shed light on T cell heterogeneity. *Carr. Opin. Immunol.* 61, 17–25 (2019).
2. Spitzer, M. H. & Nolan, G. P. Mass cytometry: single cells, many features. *Cell* 165, 780–791 (2016).
3. Peterson, V. M. et al. Multiplexed quantification of proteins and transcripts in single cells. *Nat. Biotechnol.* 35, 936–939 (2017).
4. Stocekius, M. et al. Simultaneous epitope and transcriptome measurement in single cells. *Nat. Methods* 14, 865–868 (2017).
5. Carr, E. J. et al. The cellular composition of the human immune system is shaped by age and cohabitation. *Nat. Immunol.* 17, 461–468 (2016).
6. Brodin, P. et al. Variation in the human immune system is largely driven by non-heritable influences. *Cell* 160, 37–47 (2015).
7. Houben, R. M. & Dodd, P. J. The global burden of latent tuberculosis infection: a reestimation using mathematical modelling. *PLoS Med.* 13, e1002152 (2016).
8. World Health Organization. *Global Tuberculosis Report 2020*. (WHO, 2020); [https://www.who.int/tb/publications/global_report/en/](https://www.who.int/tb/publications/global_report/en/).
9. Verrier, S. et al. Rate of reinfection tuberculosis after successful treatment is higher than rate of new tuberculosis. *Am. J. Respir. Crit. Care Med.* 171, 1430–1435 (2005).
10. Day, C. L. et al. PD-1 expression on M. tuberculosis-specific CD4 T cells is associated with bacterial load in human tuberculosis. *Front. Immunol.* 9, 1995 (2018).
11. Adekambi, T. et al. Distinct effector memory CD4+ T cell signatures in latent *Mycobacterium tuberculosis* infection, BCG vaccination and clinically resolved tuberculosis. *PLoS ONE* 7, e36046 (2012).
12. Arrigucci, R. et al. Active tuberculosis characterized by highly differentiated effector memory Tγ1 cells. *Front. Immunol.* 9, 2127 (2018).
13. Lindestam Arlehamn, C. S. et al. Memory T cells in latent *Mycobacterium tuberculosis* infection are directed against three antigenic islands and largely contained in a CXCR3+CCR6+ Tγ1 subset. *PLoS Pathog.* 9, e1003150 (2013).
14. Scriba, T. J. et al. Distinct, specific IL-17- and IL-22-producing CD4+ T cell subsets contribute to the human anti-mycobacterial immune response. *J. Immunol.* 180, 1961–1970 (2008).
15. Coulter, F. et al. IL-17 production from T helper 17, mucosal-associated invariant T, and γδ cells in tuberculosis infection and disease. *Front. Immunol.* 8, 1252 (2017).
16. Bred, J. G. et al. Transcriptomic analysis of CD4+ T cells reveals novel immune signatures of latent tuberculosis. *J. Immunol.* 200, 3283–3290 (2018).
17. Perreau, M. et al. Lack of *Mycobacterium tuberculosis*-specific interleukin-17A-producing CD4+ T cells in active disease. *Eur. J. Immunol.* 43, 939–948 (2013).
18. Recuera, M. C. et al. Transmissibility and potential for disease progression of drug resistant *Mycobacterium tuberculosis* prospective cohort study. *BMJ* 367, i5894 (2019).
19. Zunila, A., Raviglione, M., Hafner, C. & von Reyn, C. F. Tuberculosis. *N. Engl. J. Med.* 368, 745–753 (2015).
20. Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* 4, 330–336 (2003).
21. Liu, W. et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ Treg cells. *J. Exp. Med.* 203, 1701–1711 (2006).
22. Castrejon-Arcelus, M. et al. Lymphocyte inattness defined by transcriptional states reflects a balance between proliferation and effector functions. *Nat. Commun.* 10, 687 (2019).
23. Korsunsky, I. et al. Fast, sensitive, and accurate integration of single-cell data with Harmony. *Nat. Methods* 16, 1289–1290 (2019).
24. Savage, A. K. et al. The transcription factor PLZF directs the effector program of the NKT cell lineage. *Immunity* 29, 391–403 (2008).
25. Suliman, S. et al. Peripheral blood mucosal-associated invariant T cells in tuberculosis patients and healthy *Mycobacterium tuberculosis*-exposed controls. *J. Infect. Dis.* 222, 995–1007 (2020).
26. Sallustro, F., Leng, D., Förster, R., Lipp, M. & Lanzavecchia, A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401, 708–712 (1999).
27. Jockel, L. T. & Bird, P. I. Are all granzymes cytotoxic in vivo? *Biochem. J.* 395, 181–202 (2014).
28. Maecder, H. T., McCoy, J. P. & Nussenblatt, R. Standardizing immunophenotyping for the Human Immunology Project. *Nat. Rev. Immunol.* 12, 191–200 (2012).
29. Acosta-Rodriguez, E. V. et al. Surface phenotype and antigenic specificity of human interleukin 17–producing T helper memory cells. *Nat. Immunol.* 8, 639–646 (2007).
30. Szabo, S. et al. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 100, 665–669 (2000).
31. Sallustro, F., Zielinski, C. E. & Lanzavecchia, A. Human TH17 subsets. *Eur. J. Immunol.* 42, 2215–2220 (2012).
32. Wambre, E. et al. A phenotypically and functionally distinct human Th2 cell subpopulation is associated with allergic disorders. *Sci. Transl. Med.* 9, eaam9171 (2017).
33. Kleinewietfeld, M. et al. CCR6 expression defines regulatory effector/memory-like cells within the CD25+CD4+ T cell subset. Blood 105, 2877–2886 (2005).
34. Lee, J. et al. Enrichment of human CCR6+ regulatory T cells with superior suppressive activity in oral cancer. J. Immunol. 199, 467–476 (2017).
35. Fonseka, C. Y. et al. Mixed-effects association of single cells identifies an expanded effector CD4+ T cell subset in rheumatoid arthritis. Sci. Transl. Med. 10, eaau0305 (2018).
36. Kaczorowski, K. J. et al. Continuous immunotypes describe human immune variation and predict diverse responses. Proc. Natl Acad. Sci. USA 114, E6097–E6106 (2017).
37. Hashimoto, K. et al. Single-cell transcriptomics reveals expansion of cytotoxic CD4 T cells in supercentenarians. Proc. Natl Acad. Sci. USA 116, 24242–24251 (2019).
38. Amadori, A. et al. Genetic control of the CD4/CD8 T cell ratio in humans. Nat. Med. 1, 1279–1283 (1995).
39. Kissick, H. T. et al. Androgens alter T cell immunity by inhibiting T-helper 1 differentiation. Proc. Natl Acad. Sci. USA 111, 9887–9892 (2014).
40. Ter Horst, R. et al. Host and environmental factors influencing individual human cytokine responses. Cell 167, 1111–1124 (2016).
41. Nedelec, Y. et al. Genetic ancestry and natural selection drive population differences in immune responses to pathogens. Cell 167, 657–669 (2016).
42. Berry, M. P. et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 466, 973–977 (2010).
43. Scriba, T. J. et al. Sequential inflammatory processes define human progression from M. tuberculosis infection to tuberculosis disease. PLoS Pathog. 13, e1006687 (2017).
44. Fritsch, R. D. et al. Stepwise differentiation of CD4 memory T cells defined by expression ofCCR7 and CD27. J. Immunol. 175, 6489–6497 (2005).
45. Coquet, J. M. et al. The CD27 and CD70 costimulatory pathway inhibits effector function of T helper 17 cells and attenuates associated autoimmunity. Immunity 38, 53–65 (2013).
46. MA State Public Health Laboratory Tuberculosis Report. (Massachusetts Department of Public Health, 2020).
47. Arlehamn, C. S., McKinney, D. M. & Carpenter, C. A quantitative analysis of complexity of human pathogen-specific CD4 T cell responses in healthy M. tuberculosis-infected South Africans. PLoS Pathog. 12, e1005760 (2016).
48. Christophersen, A. et al. Distinct phenotype of CD4+ T cells driving celiac disease identified in multiple autoimmune conditions. Nat. Med. 25, 734–737 (2019).
49. Zhang, F. et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. Nat. Immunol. 20, 928–942 (2019).
50. Smillie, C. S. et al. Intra- and inter-cellular rewiring of the human colon during ulcerative colitis. Cell 178, 714–730 (2019).
51. Boisson-Dupuis, S. et al. Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. Immunol. Rev. 264, 103–120 (2015).
52. Pai, M. et al. Tuberculosis. Nat. Rev. Dis. Primers 2, 16076 (2016).
53. Bustamante, J., Boisson-Dupuis, S., Abel, L. & Casanova, J. L. Mendelian susceptibility to mycobacterial disease: genetic, immunological and clinical features of inborn errors of IFN-γ immunity. Semin. Immunol. 26, 454–470 (2014).
54. Okada, S. et al. Impairment of immunity to Candida and Mycobacterium in humans with bi-allelic RORC mutations. Science 349, 606–613 (2015).
55. Luo, Y. et al. Early progression to active tuberculosis is a highly heritable trait driven by 3q23 in Peruvians. Nat. Commun. 10, 3765 (2019).
56. Cosmi, L. et al. Human interleukin 17–producing cells originate from a CD161+CD4+ T cell precursor. J. Exp. Med. 205, 1903–1916 (2008).
57. Bengsch, B. et al. Human Tp,17 cells express high levels of enzymatically active dipeptidylpeptidase IV (CD26). J. Immunol. 188, 5458–5447 (2012).
58. Morimoto, C. & Schlossman, S. F. The structure and function of CD26 in the T cell immune response. Immunol. Rev. 161, 55–70 (1998).
59. Fergusson, J. R. et al. CD161 defines a transcriptional and functional phenotype across distinct human T cell lineages. Cell Rep. 9, 1075–1088 (2014).
60. Yamazaki, T. et al. CCR6 regulates the migration of inflammatory and regulatory T cells. J. Immunol. 181, 8391–8401 (2008).
61. Scriba, T. J. et al. Differential recognition of Mycobacterium tuberculosis-specific epitopes as a function of tuberculosis disease history. Am. J. Respir. Crit. Care Med. 196, 772–781 (2017).
62. Chowdhury, R. et al. A multi-cohort study of the immune factors associated with pulmonary tuberculosis disease. Scientific Rep. 6, 28586 (2016).
63. Khader, S. A. et al. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. Nat. Immunol. 8, 369–377 (2007).
64. Arlehamn, C. L. et al. Transcriptional profile of tuberculosis antigen-specific T cells reveals novel multifunctional features. J. Immunol. 193, 2931–2940 (2014).
65. Shaamugasundaram, U. et al. Pulmonary Mycobacterium tuberculosis control associates with CXCR3- and CCR6-expressing antigen-specific Th1 and Th17 cell recruitment. JCI Insight 5, 137858 (2020).
66. Darrah, P. A. et al. Prevention of tuberculosis in macaques after intravenous BCG immunization. Nature 577, 95–102 (2020).
67. Lindenstrom, T. et al. Vaccine-induced Tp,17 cells are maintained long-term postvaccination as a distinct and phenotypically stable memory subset. Infect. Immun. 80, 3533–3544 (2012).
68. Fares, A. Seasonality of tuberculosis. J. Glob. Infect. Dis. 3, 46–55 (2011).

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Methods
Clinical cohort. Our cohort is a subset of 18,544 individuals from a large epidemiological parent study of risk factors for TB infection and disease conducted between 2008 and 2012 (protocol approved by the Harvard University Institutional Review Board [IRB no. 39332] and the Research Ethics Committee of the National Institute of Health of Peru). All study participants were recruited from 106 district health centers in Lima, Peru and provided written informed consent. We enrolled index patients aged 16 or older with microbiologically confirmed pulmonary TB. Within 2 weeks of enrolling an index patient, we enrolled their household contacts who were assessed for co-prevalent TB disease by clinical evaluation and for Mtb infection by a TST. Household contacts were reassessed at 2, 6 and 12 months for evidence of new Mtb infection or TB disease. At the time of enrollment, we collected demographic, health and socioeconomic data from both index patients and household contacts, including age, sex, height, weight, alcohol use, smoking, prior incarceration, BCG vaccination scars, intensity of previous TB diagnosis (Supplementary Table 1). Nutritional status was determined for children (age ≤ 19) based on the World Health Organization BMI z-score tables and for adults based on BMI thresholds: underweight (children: z-score ≤ −2; adults: BMI < 18.5), normal weight (children: −2 < z-score ≤ 2; adults: 18.5 ≤ BMI ≤ 25) or overweight (children: z-score > 2; adults: BMI ≥ 25). Alcohol use was categorized as nondrinker (0 alcoholic drinks per day), light (<40 g of alcohol or <3 alcoholic drinks per day) or heavy (≥40 g of alcohol or ≥3 alcoholic drinks per day). Smoking was categorized as nonsmoker (0 cigarettes per day), light (1 cigarette per day) or heavy (>1 cigarette per day). BCG vaccination status was self-reported. The number of BCG scars was based on physician’s observation. SES was quantified into tertiles from a principal-component analysis (PCA) that included type of housing, access to a water supply and sanitation. Season of blood draw was classified based on local temperatures: winter (June–September), spring (October–December) and summer (January–May).
For this study, we re-consented and enrolled a subset of 264 participants whom we visited to obtain information on their TB history subsequent to the completion of the parent study and to obtain PBMCs. We considered index patients and household contacts who developed TB disease during follow-up as cases, and TST− household contacts who did not develop TB disease as controls. We excluded participants if they did not consent to reenrollment or were positive for human immunodeficiency virus. We collected blood at a median of 5.7 years after enrollment in the parent study (range 4.72–6.60 years). All cases had been infected with drug-sensitive strains and received treatment before re-recruitment. Controls were excluded if they were first-degree relatives of their index patient. We calculated associations between each covariate and TB disease status with either a two-sided t-test (continuous covariates) or a chi-squared test (categorical covariates). For significantly associated covariates, we reestimated associations in a multivariate logistic regression model and determined significance based on coefficient P values.
Sample processing. PBMC sample preparation. Within 6–8 h of obtaining blood samples, we purified PBMCs using Ficoll–Hypaque centrifugation as described previously, followed by cryopreservation at a concentration of 5 million cells per ml for shipping to Boston.
We quickly thawed cryopreserved PBMCs (10 million cells) and added each sample dropwise to prewarmed complete RPMI (cRPMI; RPMI 1640 medium (Lonza) supplemented with 5% FBS (Gemini Bio), 5 μM 2-mercaptoethanol (Gibco), 200 U/ml penicillin–streptomycin (Gibco) and 100 U/ml of 1% UltraPure BSA for 10 min at 37 °C. We then added a TotalSeq-A (BioLegend) oligonucleotide-labeled antibody mix (anti-CD45RA suspended in 10 μl of labeling buffer) and incubated all samples at room temperature for 25 min. Next, we stained cells with the remaining 30 TotalSeq-A antibodies (Supplementary Table 2) for 25 min at 4 °C and washed cells three times with 2 ml of 1% BSA in labeling buffer sequentially. Each sample was passed through a 40-μm filter and kept on ice before sorting on a BD FACSAria Fusion cell sorter.
We sorted up to 10,000 live cells from each sample based on forward and side scatter gating to remove non-lymphocytes, dead cells and other impurities. We then pooled cells into batches of six donors. Batch assignments were randomized, requiring that no two donors in the same batch had a relatedness estimation in admixed populations (REAP) kinship score greater than 0.125 (at most, second cousins) based on genotype to facilitate post hoc demultiplexing. Pools of six samples were sorted into one microcentrifuge tube prepared with 200 μl of 0.04% BSA in PBS, and each pool was processed as one single-cell RNA-seq sample.
We prepared mRNA and surface re-arranger libraries for each batch at the Brigham and Women’s Hospital Single Cell Genomics Core using the Chromium Single Cell 3’ v3 kit (10x Genomics). Pairs of libraries prepared on the same day were pooled and sequenced to a depth of 400 million reads per lane on an Illumina HiSeq X with paired-end 150 base-pair reads. In total, we sequenced 276 samples across 46 pooled libraries in six sequencing runs.
Bulk RNA sequencing. PBMCs were thawed in warm cVIVO15 (X-VIVO 15 medium (Lonza) supplemented with 5% FBS (Gemini Bio), 5 μM 2-mercaptoethanol (Gibco) and 10 mM N-acetyl-L-cysteine (Sigma)). We washed cells twice with cVIVO15, resuspended in PBS and spun down. Cell pellets were resuspended in RLT Plus buffer (Qiagen) and flash frozen on dry ice.
For RNA isolation, we thawed, vortexed and incubated samples for 5 min at room temperature before RNA isolation using the Qiagen RNasey extraction kit.
After isolation, libraries were generated at the Molecular Biology Core Facilities at the Dana–Farber Cancer Institute and sequenced on an Illumina NovaSeq.
Genotyping and genetic data processing. We genotyped all individuals on the LIMAarray, a previously described custom Affymetrix array design based on whole-exome sequencing from 116 Peruvian individuals with active TB. Genotypes were called for all 4,002 individuals in the original genetic study with the apt-genotype-axiom program. We excluded individuals with high genotype missingness (≥5% of loci) or a high heterozygosity rate (≥5 standard deviations). We excluded loci that had a significant association with batch (P < 1 × 10−6), a low call rate (<95%), a large difference in per-single-nucleotide polymorphism (SNP) missingness rate between cases and controls (>0.1), a Hardy–Weinberg P value below 10−14 in controls and duplicated position markers. After individual- and SNP-level QC, there were 263 donors and 677,385 SNPs remaining.
To measure global genetic ancestry proportions for each donor, we joined our cohort with previously published genotypes from the 1000 Genomes Project phase 3 (2,054 individuals from 26 populations), Siberians (245 individuals from 17 populations) and Native Americans (493 individuals from 57 populations) based on variant-level matching. After removing variants with minor allele frequency <0.001 and excluding genotypes with PCA scores below the median for each population, we used ADMIXTURE (version 1.3) at K = 4 (ref. 41). After the admixed nature of the cohort, we calculated an ancestry-aware genetic relatedness matrix with the REAP kinship score to account for LD differences.
We pre-phased genotypes with SHAPEIT2 and imputed genotypes at untyped autosomal loci with IMPUTE2, using the 1000 Genomes Project Phase 3 dataset as a reference panel. After removing SNPs with INFO scores of less than 1, 738,194 SNPs remained.
Statistical analysis of genomic data. Aligning single-cell data and quantifying features. We used Cell Ranger (version 3.1.0) to conduct all alignment and feature quantification of multimodal single-cell sequencing data. For mRNA, we aligned reads to the human genome (GRCh38 for transcriptomic analysis and hg19 for genotype-based demultiplexing). We aligned surface protein reads to a dictionary of feature tags. We collapsed reads mapping to the same UMI in the same cell.
Single-cell sample demultiplexing. We demultiplexed the six samples within each pooled batch based on genotypes at 7,378,194 SNPs. We used DeMUXlet with default parameters, removed cells with ambiguous or doublet assignments and verified our accuracy by correlating the number of cells demultiplexed per sample with the number of live cells sorted after memory T cell isolation.
Single-cell sequencing data quality control. We removed cells that expressed fewer than 300 genes or had more than 20% of their UMIs mapping to MT genes.
Gene expression UMI counts were normalized per cell for library size and log-transformed:

\[
\log_{\text{total counts}} \left( \text{feature counts} \times 10,000 \right)
\]

For samples with high live cell counts but low numbers of demultiplexed cells, we merged single-cell sequencing reads assigned to each donor and called variants from merged data using bcftools (v1.9)\(^79\). We quantified the concordance between sequencing-based genotypes and array-based genotypes and corrected the donor labels for four samples. We identified and removed an additional four mislabeled samples. One more sample was removed for high genotype missingness and heterozygosity rate.

We normalized the surface marker UMI counts using a centered log-ratio transformation for each cell. We used an in silico gating strategy to identify and remove contaminating non-memory T cells: we binned cells based on their normalized expression of CD3 and CD45RO, manually determined thresholds of each marker’s expression to separate discrete subpopulations and removed cells with expression of either marker below those thresholds. We additionally removed cells that were in clusters dominated by non-memory T cells.

**Unimodal pipeline for dimensionality reduction.** For each modality, we selected the union of the top 1,000 features with highest variance in each library preparation pool, and scaled the expression of each feature across all cells to have a mean of 0 and a variance of 1. For the mRNA expression, we also cosine normalized the scaled expression values. We used truncated PCA (trPCA R package) to reduce the data into 20 dimensions and then corrected these principal components for donor and library preparation batch effects using Harmony\(^23\). With UMAP, we reduced the batch-corrected embeddings into two dimensions for visualization\(^20\).

**Multimodal pipeline for dimensionality reduction.** For each modality, we selected the union of the top 1,000 features with highest variance in each library preparation pool and scaled the normalized expression of each feature across all cells to have a mean of 0 and a variance of 1. We excluded TCR genes because of potential mapping errors due to recombination and sequence similarity. Then, we used CCA as implemented in the cc function (CCA R package) to calculate canonical dimensions. This method finds maximally correlated linear combinations of features from each modality, that is, calculates vectors \(a\) and \(b\) for mRNA matrix \(X\) and surface protein matrix \(Y\) to maximize \(\text{corr}(Xa, Yb)\) subject to the constraint that \(\text{var}(Xa) = \text{var}(Yb) = 1\). We defined CVs by projecting cells onto each canonical dimension in the mRNA space (CV1 = \(Xa\)) and selecting the top 20 dimensions defined by highest canonical correlations \(\text{corr}(Xa, Yb)\) between mRNA and protein. We corrected donor and batch effects with Harmony and reduced the batch-corrected embeddings into two dimensions with UMAP.

**Clustering and annotating cell states.** Cells were clustered based on low-dimensional embeddings (principal components or CVs). We constructed a shared nearest-neighbor graph (Seurat R package) and conducted Louvain modularity clustering at a range of resolutions. Results are shown at a resolution of 2.00, which yielded 31 CCA-based clusters with at least ten cells from more than five donors.

To annotate clusters as cell states, we identified pseudo-bulk differentially expressed mRNA and surface protein features between cells inside and outside of each cluster. We collapsed single-cell expression profiles for each modality into pseudo-bulk profiles by summing the raw UMI counts for each gene or surface protein across all cells from the same donor, batch, and cluster. For mRNA, we limited differential expression analysis to genes that had at least 30 UMIs detected in at least 120 pseudo-bulk samples (\(n = 4,540\), and for both modalities, we normalized counts for each feature in each pseudo-bulk sample into counts per million (CPM)). We used separate linear models to estimate the effect of each cluster on pseudo-bulk expression of each gene, accounting for donor, batch and the number of UMIs assigned to each pseudo-bulk sample. \(P\) values were obtained through an LRT between the models with and without the cluster term. We considered a gene or surface protein to be a marker of a cluster if it had a \(P < 0.05\) (4,540 genes \(\times 31\) clusters) \(\times 3.6\times 10^{-2}\) and a fold change > 2. We manually annotated each cell state based on the literature.

**Aligning bulk RNA-seq data and quantifying features.** We used STAR (version 2.7.6a) to conduct all alignment of bulk RNA-seq data\(^82\). Paired-end reads were aligned to the human transcriptome (GRCh38), and we quantified gene-level heterozygosity rate.

Constructing a linear predictor of \(C_{12}\) abundance. We trained a linear model on pseudo-bulk T cell and bulk PBMC training data from our cohort to estimate the abundance of \(C_{12}\) based on bulk RNA expression profiling data. For \(T\) cells, we comprised pseudo-bulk expression of each gene in each sample (\(n = 271\), including 12 technical replicates) by summing gene counts across all cells from that sample, removing genes with <30 counts in any sample and normalizing these measurements within each sample to log2(CPM). For each sample, we computed the proportion of cells in \(C_{12}\) out of all memory T cells passing QC in the CITE-seq dataset, and we measured bulk gene expression in PBMCs from a subset (\(n = 15\)) of the \(T\) cell samples chosen to maximize variation of \(C_{12}\) proportions. Genes with average expression <2 were removed, and we normalized each gene to log2(transcripts per million).

The predictor was a linear model with a ridge regularization term to avoid overfitting. We used tenfold cross-validation to compute the optimal value of the ridge parameter (\(\lambda = 0.0002\)). We used the glmnet R package to train a ridge regression model with this parameter to predict the proportion of \(C_{12}\) cells in each sample based on gene expression\(^19\). Features were limited to the 2,355 genes at the intersection of most variable genes and genes measured in the training and target dataset for prediction. To evaluate accuracy, we used tenfold cross-validation to train the model on 90% of samples and predict \(C_{12}\) abundance in the remaining 10% of samples, and computed the Pearson correlation \(r\) between predicted and actual abundance. These predicted abundances in LIMAA samples were later used for comparison with predicted abundances in other cohorts.

We obtained RNA microarray data from Berry et al. from the Gene Expression Omnibus (GEO: GSE19439 and GSE19435)\(^40\). These samples were collected as part of a multicohort adult cohort recruited in the United Kingdom. Active cases were diagnosed by culture of sputum of bronchoalveolar lavage, latently infected donors were asymptomatic and positive by TST and interferon-gamma release assay (IGRA), and healthy controls had no exposure to TB and were negative by TST and IGRA. Data were preprocessed as described in the original paper, and we used the trained linear predictor to estimate the abundance of \(C_{12}\) in each...
donor. One dataset contained three groups of donors: BCG-vaccinated healthy uninfected donors, active TB donors and donors with latent infection. The other dataset contained a subset of active TB donors followed longitudinally during anti-myobac terial treatment (0, 2, and 12 months), BCG-vaccinated healthy controls. We compared C-12 proportions between pairs of donor groups within each dataset using a two-sided t-test.

To compute our power to detect the 20% case–control difference from LIMAA in C-12 in the sample by Berry et al., we repeatedly downsam pled the LIMAA memory T cell dataset to 7 cases (progressors) and 12 controls (non-progressors) 1,000 times. In each trial, we computed a P-value for the difference between the average C-12 proportion in cases versus controls with a two-sided t-test. Then, we calculated power at significance level α = 0.05 as the proportion of trials with P < α. We also obtained RNA-seq data from Scriba et al. from the GEO (GSE103147) and selected unstimulated T cell samples from two pre-disease progression time points. These samples were nested from a prospective longitudinal cohort of asymptomatic M.tb infected South African adolescents (aged 12–18 years), who were monitored for 2 years for incident TB disease. All donors (n = 54) were infected with M.tb before sample collection and either progressed to active TB at time point 0 (microbiologically confirmed; n = 25) or remained latently infected (IGRA- and/or TST positive controls; n = 29). Controls were matched for age and sex. Each donor was sampled at 1–2 time points for a total of 98 samples. Data were normalized in each sample to log2(CPM), and we used the trained linear predictor to estimate the C-12 proportion in each sample. To compute the difference in C-12 between cases and controls, accounting for repeated measures, we used a linear mixed model correcting for age, sex, sequencing technology and donor frequency between cases and controls, estimating the C-12 proportion in each sample. To compute the difference in C-12 proportion after each round of stimulation, we used a classification and regression tree model implemented with the rpart R package.

After sorting, we resampled each sample in CRPMI, counted using a Countess II Automated Cell Counter and plated each sample population at 125,000–250,000 cells per well in a 96-well round-bottom plate. We stimulated one well per population for each sample with an equal volume of CRPMI containing a 1:1 ratio of washed CD3/CD28 Dynabeads (Thermo Fisher, 111310) or CRPMI for non-stimulated controls. All conditions were incubated overnight at 37°C.

After incubation, we transferred the supernatant from each well into a new 96-well round-bottom plate and froze the plate at −20°C. We followed the manufacturer’s instructions for the LEGENDplex Human Tβ Panel (13-plex) kit (BioLegend, 740722) in a 96-well round-bottom plate. We measured the LEGENDplex signal as a 10–12.5 million cells per ml in a 96-well round-bottom plate and incubated samples overnight at 37°C. After resting the cells, we recombined all wells per donor and aliquoted 800,000–1,000,000 cells into new wells for stimulation. We stimulated cells with an equal volume of 2× PPM (81 nM PMA, 1.34 μM monomycin and 5 μM brefeldin A) and kept the remaining wells unstimulated by adding only an equal volume of 2× brefeldin (5 μg/ml); BioLegend). We incubated plates at 37°C for 4 h.

After stimulation, we transferred the samples to FACS tubes and washed twice with 500 μl CRPMI. We resuspended the samples in 500 μl of blue fluorescent live/dead cell dye with PBS (1:10,000 dilution; Invitrogen) for 20 min at room temperature. After washing, we resuspended in 50 μl of the first antibody master mix consisting of Brilliant Stain Buffer (BD Biosciences) and CCR6-PE/Cy7 (BioLegend, clone G03409). We covered each sample in foil and incubated for 25 min at room temperature. Then we added 50 μl of a second antibody master mix consisting of Brilliant Stain Buffer (BD Biosciences) and 11 surface markers (Supplementary Table 10). We incubated the samples for 25 min at 4°C. After staining, we washed the samples twice in MACS buffer.

Next, we followed manufacturer instructions to fix and permeabilize the samples using a Cyto-Fast Fix/Perm Buffer Set from BioLegend (426803). We divided the mixture of unstained cells and one tube of stimulated cells per donor with an equal volume of 2× PPM, incubated for 25 min at 37°C. After washing, we resuspended the samples in 50 μl of a second antibody master mix consisting of Brilliant Stain Buffer (BD Biosciences) and 11 surface markers (Supplementary Table 10). We incubated the samples for 25 min at 4°C. After staining, we washed the samples once in MACS buffer.

To compare cytokine production across CD4+ T cell subsets, we grafted all CD4+ T cell samples from the Peruvian cohort matched for age (<5 years), sex, season of blood draw and ascertainment of European genetic ancestry (<0.05). We counted all cells per condition and, after extracellular staining, we stained all samples with anti-IL-17A–APC (BioLegend, clone BL168) and PE dye conjugated to anti-IL-4, anti-IFN-γ, anti-IL-2, anti-IL-5, anti-IL-9, anti-IL-10, anti-IL-13, anti-IL-17F, anti-IL-21, anti-IL-22 or anti-TNF in the provided wash buffer (Supplementary Table 10c). Samples were covered in foil and incubated for 20 min at room temperature. Samples were washed twice in 1 ml of wash buffer and run on a BD LSRFortessa. Data were analyzed using FlowJo version 10.6.2. The gating structure is shown in Supplementary Fig. 3.

In two subsequent experiments, we repeated the intracellular staining experiment above using 5 million PBMCs from eight pairs of cases and controls from the Peruvian cohort matched for age (<5 years), sex, season of blood draw and ascertainment of European genetic ancestry (<0.05). We counted all cells per condition and, after extracellular staining, we stained all samples with anti-IL-17A–APC (BioLegend, clone BL168) and anti-IL-22–PE (BioLegend, clone 2G12A41). We collected data using two BD LSRFortessa analyzers.

To compare cytokine production across CD4+ T cell subsets, we grafted all CD4+ T cell samples from the Peruvian cohort matched for age (<5 years), sex, season of blood draw and ascertainment of European genetic ancestry (<0.05). We counted all cells per condition and, after extracellular staining, we stained all samples with anti-IL-17A–APC (BioLegend, clone BL168) and anti-IL-22–PE (BioLegend, clone 2G12A41). We collected data using two BD LSRFortessa analyzers.

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a concentration of 2 million cells per well. The next day, cells were recovered, counted and cultured in 96-well U-bottom plates at a concentration of 1 million cells per well. We added LEAF-purified anti-CD28 (BioLegend, CD28.2), anti-CD40 (BioLegend, HB14) and anti-CD49d (BioLegend, 9F10) to each well to a final concentration of 1 μg ml⁻¹. To the peptide-stimulated well, we added MTB300 megapool (reagent provided by C.S.L.A.) to a final concentration of 5 μg ml⁻¹ and 2μM, respectively, and incubated cells for 16–18h in a tissue culture incubator. After stimulation, cells were recovered, transferred to 96-well V-bottom plates, stained with conjugated antibodies, fixed and permeabilized, stained with antibodies for intracellular IL-17A and IFN-γ and analyzed on a BD LSRFortessa as previously described in the PMA and ionomycin stimulation experiment. We also used the same antibodies and concentrations as in that experiment (Supplementary Table 10b,c). To assess M. tuberculosis response across all T cells, we gated CD3⁺ live cells, and for C-12 cells, we gated CD4⁺CD45RO⁺CD26⁻CD161⁺CCR6⁻ Tₘ cells. For both analyses, we quantified the percentage of cells producing IL-17A or IFN-γ in the unstimulated and stimulated samples from each donor, and calculated a fold change in the mean percentage of cytokine-producing cells (across donors) between stimulated and unstimulated cells. P values were obtained from the two-sided Wilcoxon signed-rank test for paired samples. To compare cytokine production between CD26⁺CD161⁺CCR6⁺ cells from Boston control donors and TB cohort donors, we computed a P value from the Wilcoxon rank-sum test.

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**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41590-021-00933-1. Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41590-021-00933-1. Correspondence and requests for materials should be addressed to S.R.

**Peer review information**

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Extended Data Fig. 1 | CITE-seq cell and feature quality. a, In silico memory T cell gating. Each cell is plotted based on its normalized surface expression of CD3 and CD45RO, measured through CITE-seq. Gates are demarcated with red dashed lines. Red cells were removed. Counts represent the number of cells in each quadrant. b, UMAP representation of gated cells. Red cells were gated out in (a) and cells clustering with them in the UMAP (shown in black in (a) and (b)) were also removed. c, Normalized CD3 and CD45RO surface protein expression. d, Number of cells per sample after QC, stratified by TB progression status. P value is from a two-sided t test comparing mean cell counts in each group. e, Pearson correlation coefficient (r) was calculated between normalized mRNA and surface protein expression for each marker across cells passing QC. r is plotted against average normalized mRNA expression for each protein. f, Each cell is plotted based on normalized expression of each marker in surface protein and mRNA, both measured through CITE-seq, with density contours. We fit a best-fit line (in blue) with a linear model.
Extended Data Fig. 2 | Comparing proportions of eight major T cell states between flow cytometry and CITE-seq. a. Average percents per population in CITE-seq vs. flow cytometry. Gates in (b). Dashed line indicates the identity line. b. For each population, proportions plotted across 259 donors. Flow cytometry gating occurred after gating T cells. CITE-seq gating occurred after isolation of memory T cells. The dashed line indicates the identity line, and we calculated Pearson correlation coefficients (r) for each state.
Extended Data Fig. 3 | Multimodal integration with canonical correlation analysis. a. Correlations for the top 20 canonical dimensions used in downstream analysis. Bars represent the Pearson correlation between mRNA and protein projections for each dimension. b. Marker correlation with canonical variates (CVs). Each marker is plotted based on its mRNA and protein correlation with CV1 (left) or CV2 (right). c. Innateness scores. UMAP is colored based on a gene expression-derived cytotoxicity score defined in Gutierrez-Arcelus, et al. d. Correlation between innateness score and CV1. Each cell is plotted based on its innateness score from (c) and its CV1 projection, and we report the Pearson correlation coefficient.
Extended Data Fig. 4 | Single-cell expression of surface proteins, measured with CITE-seq. Each cell is colored according to its expression of each protein and plotted in UMAP space. Colors are scaled independently for each marker from minimum (blue) to maximum (yellow) expression.
Extended Data Fig. 5 | Technical replicate consistency. For each of the 31 multimodal clusters, we plotted its proportion in replicate 1 and in replicate 2 of each donor. We calculated the Pearson correlation coefficients (r) for each cluster.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Effects of donor covariates on memory T cell states. Effects of age, sex, winter blood draw and proportion of European genetic ancestry in (a) model correcting for technical covariates (# UMIs/cell, % MT UMIs/cell), donor, batch and TB disease status, and (b) full model with TB disease status, age, sex, winter blood draw, proportion of European genetic ancestry, technical covariates, donor and batch. For all, n = 271 samples from 259 independent donors. For each cluster, data are presented as the MASC OR of a cell being in each cluster given the contrast covariate (95% CI error bars) and the -log(LRT p value) of the association. The dashed horizontal line corresponds to a Bonferroni p-value threshold of 0.05/31. Labeled clusters are significant at this threshold.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Unimodal clusters and associations with TB disease progression. a–c, mRNA clusters. d–f, protein clusters. a and d, UMAPs colored by unimodal clusters. Clusters boxed in red are CD4⁺, purple are mixed CD4⁺ and CD8⁺, blue are CD8⁺, and green are CD4⁻CD8⁻. b and e, Expression of major lineage-defining surface markers measured through CITE-seq. The UMAPs are colored by the expression of five markers measured through CITE-seq. Colors are scaled independently for each marker from minimum (blue) to maximum (yellow) expression. c and f, Heatmap of overlap between unimodal and multimodal clusters. Colors indicate the proportion of the multimodal cluster (column) overlapping with the unimodal cluster (row). g, Associations between TB disease status and unimodal protein clusters. For each cluster, the data are presented as MASC ORs of a cell being in each cluster for cases vs. controls (95% CI error bars), and the -\log(LRT p value) of the association (n = 271 samples from 259 independent donors). The dashed horizontal line corresponds to a Bonferroni p-value threshold of 0.05/40. Labeled clusters are significant at this threshold. h, Abundance of C-20 in 128 cases and 131 controls. P value is from an LRT with 1 d.f. Boxplots show the median (vertical bar), 25th and 75th percentiles (lower and upper bounds of box, respectively), and 1.5xIQR (or minimum/maximum if they lie within that range; end of whiskers).
Extended Data Fig. 8 | Cell state signature extrapolation in public data. **a,** Correlation between actual and predicted C-12 proportion, per sample in memory T cell CITE-seq study (n = 271, black) and bulk PBMC RNA-seq (n = 15, blue). Line represents the identity line and we calculated the Pearson correlation coefficients (r) for T cell samples and bulk PBMC samples separately. Predicted **b** C-12 or **c** C-11 (T_{h2}, negative control) proportion in 3 categories of donors from Berry, et al. **d,** Predicted C-12 proportion in active donors at 3 time points during anti-mycobacterial treatment (0, 2, 12 months) and uninfected controls. In **b–d,** we calculated p values with a two-sided t test. **e,** Histogram of two-sided t test p values from 1,000 trials of downsampling LIMAA cohort to 7 cases and 12 controls (as in Berry, et al.) and comparing the average C-12 proportion in cases vs controls. Dashed line is the significance threshold of p = 0.05 (power = 0.15). **f,** Predicted C-12 proportion in active cases and latent controls at 2 pre-disease-progression time points in Scriba, et al. **g,** Predicted C-12 proportion in active cases and latent controls in pre- and post-disease cohorts. Pre-disease data are aggregated across 2 time points. In **f** and **g,** p-values are from a one-sided t-test (Satterthwaite’s d.f. method) of the beta estimate for TB progression status in the linear mixed model. **h,** Concordance of Pearson correlations between each cluster’s proportion and the C-12 score or the C-12 cluster’s proportion. Pearson correlation coefficients were computed for each cluster across 271 memory T cell samples. Each point represents one of the 31 clusters, and the dashed line is the identity line. All boxplots show the median (vertical bar), 25th and 75th percentiles (lower and upper bounds of box, respectively), and 1.5xIQR (or minimum/maximum if they lie within that range; end of whiskers).
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Cytokine production in Boston donors. Bars represent the mean and error bars show standard error of the mean across 5 Boston donors unascertained for TB. **a**, Per-donor percent of cells producing each cytokine in gated populations. **b**, Per-donor percent of total cytokine-producing memory CD4+ T cells in each gated population.
Extended Data Fig. 10 | *M. tb* antigen-specific response in CD4+CD26+CD161+CCR6+ memory T cells. a, Biaxial plots showing representative gating of CD3⁺CD4⁺CD45RO⁺CD161⁺CCR6⁺CD26⁺ cells in a Boston donor. b, Intracellular staining for IL-17A and IFNγ in a Boston control donor and two Peruvian TB cohort donors after either no peptide stimulation (control) or stimulation with the MTB300 megapool. c, IL-17A or IFNγ response to MTB300 stimulation in all CD3+ T cells from either Boston control donors (n = 2) or Peruvian TB cohort donors (n = 6). Each point corresponds to the percent of cells producing IL-17A or IFNγ from one donor, measured with intracellular cytokine staining. Lines connect measurements from the same donor before and after stimulation with MTB300 peptide megapool. Boxplots show the median (vertical bar), 25th and 75th percentiles (lower and upper bounds of box, respectively), and minimum/maximum (end of whiskers). P values are from a two-sided Wilcoxon signed-rank test comparing donors before and after antigen stimulation.
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☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
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☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | Flow cytometry data was collected with FACSDiva (version 8.0.1). Samples were genotyped using the customized Affymetrix UMAArray (Luo, et al, Nature Communications, 2019). Genotypes were called in a total of 4,002 samples using apt-genotype-axiom (Affymetrix). |
| Data analysis   | All data was analyzed with open-source software available online and detailed in the Methods: Flowjo (version 10.6.2), PLINK (version 1.90b3w), ADMIXTURE (version 1.3), SHAPEIT2 (version v2.r837), IMPUTE2 (version 2.3.2), Cell Ranger (version 3.1.0), Dexamet (version 1.0), bcftools (version 1.9), R (version 3.4.0), LEGENDplex data analysis software. R packages: seurat, harmonie, rliba, ggplot2, CCA, uwot, MASC, Im4e, presto, singlecellmethods, rpert, ImageTest, glmnet. Additional scripts for statistical analysis are available on GitHub (https://github.com/immunogenomics/TA_Tcell_CITEseq) |

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CITE-seq single-cell expression data (Fig. 1, 2, 5, Supplementary Fig. 1, 2, 5, 9, 11) are available on GEO (GSE158769), with sequencing data on dbGaP. Genotype data is available on dbGAP [accession: phs002025.v1.p1].
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Life sciences study design

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| Sample size | Sample size (n = 264) was determined based on number of participants re-consenting. No sample-size calculation was conducted ahead of re-recruitment due to the nature of the study. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | During re-recruitment, we used the following pre-established exclusion criteria: 1. cases infected with drug-resistant strains 2. HIV-positive participants 3. controls whose index case was a first-degree relative During single-cell data QC, we excluded samples with: 1. high genotype missingness (≥5% of loci) or high heterozygosity rate (≥3 standard deviations) 2. low concordance between sequencing-based genotype and array-based genotype for any donor |
| Replication | CITE-seq: 1. Conducted flow cytometry-based measurement of surface markers on an additional aliquot of the same samples, gated eight major populations based on surface marker expression in CITE-seq or flow cytometry, measured Pearson correlation in population frequencies between the two platforms 2. For 12 donors, ran two technical replicates each (in separate batches), and measured Pearson correlation of the frequency of each cluster across donors. All attempts at replication were successful. Functional characterization in unascertained Boston donors: 1. LEGENDplex assay: 3 donors (biological replicates) and 2 technical replicates per donor, replicated results with PMA/ionomycin stimulation with intracellular staining for the same cytokines 2. Intracellular staining: 5 donors (biological replicates) All attempts at replication were successful. Functional characterization in Peruvian donors with history of TB infection: 16 donors (biological replicates) |
| Randomization | TB progression cases and controls were randomly sampled from the larger epidemiological cohort (described in Becerra, et al. 2019). Cases were donors who had been diagnosed with active TB at initial recruitment by sputum smear (and subsequently treated successfully), and controls were household contacts who were TST+ at initial recruitment and did not subsequently develop active TB. We did not control for covariates during recruitment because we wanted to recruit a random sample. Associations with demographic covariates in our cohort are consistent with the larger epidemiological cohort, confirming that random sampling was successful. We later controlled for T cell-relevant covariates in post hoc analysis |
| Blinding | No blinding was performed because we did not assign treatment groups |

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| X | X | Eukaryotic cell lines |
| X | | Palaeontology |
| X | | Animals and other organisms |
| X | X | Human research participants |
| X | | Clinical data |
| X | X | Involved in the study |
| | X | ChIP-seq |
| | X | Flow cytometry |
| | X | MRI-based neuroimaging |
Antibodies used

Antibodies used for CITE-seq:
Antibody - Clone, Barcode, Vendor, Catalogue #, Titration (μg)
anti-CD3 (CD3E) - UCHT1, CTCAATTGAACCTCT, Biolegend, 300475, 0.5
anti-CD4 - RPA-T4, TGTCCCCGCTCAACT, Biolegend, 300563, 0.25
anti-CD5 - UCHT2, CATTACCGGAGTGC, Biolegend, 300635, 0.25
anti-CD8a/CD8B - RPA-T8, GGGCGGCTTTCCATT, Biolegend, 301067, 0.125
anti-TCR α/β (TRAC/TRBC) - IP-26, CTAACGTAGACCGA, Biolegend, 306737, 0.5
anti-CD294 (CRTL2, PTGDR2) - BM16, TGTCTTACGAGCCCC, Biolegend, 350127, 0.5
anti-CD161 (Kp.R1) - HP-3G10, GTGAGCAGTCTCTCT, Biolegend, 339945, 0.25
anti-CD25 (IL2RA) - BC56, TTTGCTTTGACTGCC, Biolegend, 302643, 0.25
anti-CD127 (IL-7R) - A019D5, GTGTTGTTCTCATG, Biolegend, 351352, 0.25
anti-CD45RO - UCHL1, CTTCGCAATCATGTTG, Biolegend, 304255, 0.5
anti-CD27 - Q323, GCACCTCTCTGATGA, Biolegend, 302847, 0.5
anti-CD62L - DREG56, GTCTCTGCAACTTGA, Biolegend, 304847, 0.25
anti-CD29 (ITG81) - TS2/16, GTATCCTTCACTCA, Biolegend, 303027, 0.25
anti-CD88 - H1T2, GTACCCGGCTTGTGA, Biolegend, 303541, 0.25
anti-CD26 (DPF4) - BA5b, GGTGACGATGATAAGT, Biolegend, 302720, 0.25
anti-HLA-DR - L243, AATAGCGAGCAAGTA, Biolegend, 307659, 0.25
anti-CD69 - FN50, GTCTCTGGCCTTAAA, Biolegend, 310947, 0.25
anti-CD28 - CD28.2, TGGAAGCAACCCCTTCT, Biolegend, 302955, 0.25
anti-CD78 (ICOS) - CD98.4A, GGCCGACCAACTTAA, Biolegend, 313555, 0.5
anti-CD244 (2B4) - CL7, TCCCTTGATGCTAG, Biolegend, 329527, 0.25
anti-CD272 (RTLA) - MH26, GTATGCTGACTAG, Biolegend, 344525, 0.5
anti-CD95 (FAS) - DX2, CCACCTCATAGGC, Biolegend, 305649, 0.25
anti-CD48 (SLAMF2, BLAST-1) - B4A, TTAGCATGAGAAA, Biolegend, 336709, 0.25
anti-CD79a (PD-1) - EH12.2H7, ACAGCGGCCATTCTTA, Biolegend, 339955, 0.25
anti-Ti6T - A151536, TGCTTCAAGCGCA, Biolegend, 372725, 0.25
anti-CD183 (CXCR3) - G025H7, GGAGATGGTATATTTAA, Biolegend, 353745, 0.5
anti-CD4 (CCR5) - L293H, AGGCCTCTGGCAAGA, Biolegend, 394973, 0.25
anti-CD195 (CCR5) - J418F3, CCAAGTGAAGACCA, Biolegend, 395195, 0.25
anti-CD196 (CCR6) - G0343, GATCCCTTTTGACT, Biolegend, 353437, 0.25
anti-CD197 (CCR7) - G034H7, AGTCACTCAACCCGA, Biolegend, 353247, 0.25
Mouse IgG1, κ isotype Ctrl - MOPC-21, CGGCGACGACATTAA, Biolegend, 400199, 0.5

Antibodies used for flow cytometry:
Antibody - Fluorophore, Clone, Vendor, Catalogue #, Volume (μl)
anti-CD3 - FITC, UCHT1, invitrogen, 11-0038-42, 0.5
anti-CD4 - BV421, RPA-T4, Biolegend, 300532, 0.2
anti-CD8a - BV711, RPA-T8, Biolegend, 301044, 0.2
anti-CD14 - AF700, MSF3, BD Pharmining, 557923, 1
anti-CD16 - BV510, 3D8, Biolegend, 302048, 0.1
anti-CD19 - APC-Cy7, HIB19, Biolegend, 302218, 0.5
anti-CD27 - PE, O373, Biolegend, 308808, 0.2
anti-CD56 - PE-Cy7, HCD56, Biolegend, 318318, 0.3
anti-CD61 - APC, DREG56, Biolegend, 304810, 0.3
anti-TCR α/β - PE Dazzle, IP-26, Biolegend, 306726, 0.75
anti-HLA-DR - BV605, L243, Biolegend, 307640, 1
anti-CD15 - BV395, H98, BD Horizon, 563872, 0.5
anti-CD14 - BV455, MOPS, BD Horizon, 563561, 1
anti-CD19 - BV455, S25C1, BD Horizon, 563549, 2.5
anti-CD3 - APC-Cy7, UCHT1, Biolegend, 300426, 1
anti-CCR6 - PE-Cy7, G03490, Biolegend, 353418, 0.5
anti-CD26 - BV605, M-A261, BD OptiBuild, 744450, 0.5
anti-CD127 - FITC, A019D5, Biolegend, 351312, 1
anti-CD45RO - BV605, UCHL1, Biolegend, 304232, 0.5
anti-CD25 - BV711, M-A251, Biolegend, 356138, 1
anti-CD62L - A700, DR656, Biolegend, 304820, 0.5
anti-CD161 - PE-Dazzle, HP-3610, Biolegend, 339940, 2
anti-L17a - APC, BL685, Biolegend, 512334, 0.5
anti-IL2 - PE, MO21-17H12, Biolegend, 500306, 1
anti-IL4 - PE, BD4-8, Biolegend, 500704, 2
anti-IL5 - PE, JES1-39D10, Biolegend, 500903, 2
anti-IL19 - PE, MH944, Biolegend, 507604, 4
anti-IL10 - PE, JES3-9D7, Biolegend, 501403, 4
anti-IL13 - PE, JES510-S2A, Biolegend, 501903, 2
anti-IL17F - PE, SHL17, Invitrogen, 12-7169-41, 1
anti-IL21 - PE, 4B6G1, Biolegend, 516703, 1
anti-IL22 - PE, 2G12A4, Biolegend, 366703, 2
anti-IFNα - PE, MAb11, Biolegend, 502908, 0.25
anti-IFNγ - PE, B27, Biolegend, 506507, 0.2
Antibodies used for antigen stimulation:
- Antibody, Clone, Vendor, Catalog #, Concentration (µg/ml)
  - anti-CD28, 29.2, BioLegend, 302933, 1
  - anti-CD40, HB14, BioLegend, 313019, 1
  - anti-CD49d, 9F10, BioLegend, 304333, 1

Validation

All commercial CITE-seq antibodies were validated for human T cell specificity over three pilot CITE-seq experiments, where we tested the quality of and appropriate titration for each antibody. All commercial flow cytometry antibodies were validated by the manufacturer, as stated. BioLegend CITE-seq: All lots are tested by flow cytometry to make sure they stain the expected cell population and that oligos are attached to the antibodies. This process has been validated by comparison with a traditional two-step flow cytometry staining as shown.

Invitrogen (anti-CD3): This Antibody was verified by Relative expression to ensure that the antibody binds to the antigen stated.

BioLegend: All newly developed clones at BioLegend undergo validation testing for multiple applications. This serves as a cross-check for specificity and provides clarity for research uses. Typically, antibodies are tested by two or more of the below methods.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Population characteristics detailed in Supplementary Table 1.

Recruitment

We re-recruited 264 individuals from a larger epidemiological study (n = 13,499) in Lima, Peru that identified patients with active TB and followed their Mtb-infected household contacts for one year to monitor progression to active disease. Index case participants in the original epidemiological study were recruited at 106 district health centers, with follow-up recruitment of household contacts through home visits. All study participants provided written informed consent. For this study, we re-consented and enrolled a subset of 264 participants whom we visited to obtain information on their TB history subsequent to the completion of the parent study. We excluded participants if they did not consent to re-enrollment or were HIV-positive. Based on this recruitment design, we do not anticipate biases influencing cohort composition.

Ethics oversight

The study protocol was approved by the Institutional Review Board of Harvard School of Public Health (Ref. No. 19332) and by the Research Ethics Committee of the National Institute of Health of Peru.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrom e used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Peruvian human peripheral blood mononuclear cells (PBMCs) were thawed from liquid nitrogen and washed. In summary, samples were stained with blue fluorescent Live/Dead fixable dead cell stain (Invitrogen) for 20 minutes at room temperature and then with anti-CD3 - FITC (UCHT1), anti-CD4 (RPA-T4), anti-CD8a (RPA-T8), anti-CD14 (M5E2), anti-CD16 (3G8), anti-CD19 (HIB19), anti-CD27 (O523), anti-CD56 (HCD56), anti-CD62L (DREG56), anti-TCR α/β (IP26), anti-HLA-DR (L243), anti-CD15 (HI98), and anti-CD45RO (UCHL1) in Brilliant Stain buffer for 25 min at 4°C. We washed samples with MACS buffer (pH 7.4 PBS, 2mMEDTA, 2% FBS) and filtered through 40 um mesh.

For validation experiments, isolated human CD4 T cells were thawed, washed, and resuspended in blue fluorescent Live/Dead fixable dead cell stain with PBS (Invitrogen) for 20 min. Next, they were stained with CCR6 - PE/Cy7 (G03409) in Brilliant Stain Buffer for 25 min at room temperature, followed by antibodies to CD14 (MOP9), CD19 (SJ25C1), CD127 (A019D5), CD4 (RPA-T4), CD26 (M-A261), CD62L (DREG56), CD25 (M-A251), CD61 (HP-3610), CD3 (UCHT1) and CD45RO (UCHL1) for 25 minutes at 4°C. After washing, we followed manufacturer instructions to fix and permeabilize the samples. We stained with an intracellular antibody master mix consisting of IL17a - APC (BL168) and PE conjugated to anti-IL-4 (8D4-8), anti-IFNγ (B27), anti-IL2 (MQ1-17H12), anti-IL5 (JES9-39D10), anti-IL9 (MH94A), anti-IL10 (JES10-9D7), anti-IL13 (JES10-5A2), anti-IL17a (SHL17), anti-IL21 (11B11), anti-IL22 (G12A41), or anti-TNF (MAb11) for 20 min at room temperature.

For Mtb antigen stimulation experiments, after stimulation human PBMCs were recovered, transferred to 96-well V bottom plates, stained with conjugated antibodies, fixed and permeabilized, stained with antibodies for intracellular IL-17A and IFNγ (same antibodies and concentrations as in prior experiment).

Instrument

We collected data from each sample on the BD LSRSort Fortessa TM and sorted samples using a BD FACS Aria Fusion Cell Sorter.
Data was collected using FACSDiva version 8.0.1 and data was analyzed with FlowJo version 10.6.2.

**Cell population abundance**

All PBMCs were analyzed in initial flow cytometry experiments. For validation experiments, CD4 T cells were isolated using a human CD4+ T cell negative isolation kit (Miltenyi biotec, Cat #130-096-533). Following manufacturer instructions, we achieved a high purity of >90% based on flow cytometry analysis.

The abundance of each population in the 4-way sort preceding our cytokine quantification assay are as follows:

1. Naive CD4 T cells (CD3+ CD4+ CD45RO- CD62L+): ~20-40%
2. Other memory CD4 T cells (CD3+ CD4+ CD45RO+ CCR6-/+ CD26-/+ CD161/-/+): ~20-40%
3. Treg (CD3+ CD4+ CD25+ CD127low): ~2-3%
4. Target population (CD3+ CD4+ CD45RO+ CCR6+ CD26+ CD161+): ~2-8%

**Gating strategy**

Peruvian PBMCs were gated into the following parent populations:

- Memory CD4 T cells: FSC/ SSC lymphocytes, Singlets, Live/dead-, CD14-, CD3+, CD45RO+, TCRab+, CD4+
- Memory CD8 T cells: FSC/ SSC lymphocytes, Singlets, Live/dead-, CD14-, CD3-, CD45RO+, TCRab+, CD8+
- Gamma delta T cells: FSC/ SSC lymphocytes, Singlets, Live/dead-, CD14-, CD3+, CD45RO+, TCRab-

In validation experiments, our target population was gated as follows: FSC/ SSC lymphocytes, Singlets, Live/dead-, CD14-, CD19-, CD3+ CD4+ CD45RO+ CCR6+ CD26+ CD161+

In Mtb antigen stimulation experiments, our target population was gated as follows: FSC/ SSC lymphocytes, Singlets, Live/dead-, CD14-, CD19-, CD3+ CD4+, non-Treg (CD25- CD127high) CD45RO+ CCR6+ CD26+ CD161+

❐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.