**Sample preparation**

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation using standard procedures (Ficoll-Paque Plus, GE Healthcare).

**Haplotype characterization**

PBMCs from patients and healthy donors were stained to determine their haplotype. The characterization was performed with 0.5 x 106 cells incubated with the antibodies for 15 minutes at 4 ° C.

 The following antibodies were used at the dilutions listed in the table below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibody** | **Clone** | **Fluorochrome** | **Titer** | **Company** |
| anti-HLA-A02 | BB7.2 | PE | 1:50 | BD Pharmingen |
| anti-HLA-B7 | BB7.1 | PE | 1:50 | Abcam |
| anti-HLA-B08 | 145 | PE-Cy7 | 1:50 | Miltenyi Biotec |

PBMC from individuals positive for at least one MHC allele were subsequently labeled with the corresponding peptide-loaded MHC-I pentamers. Some individuals tested positive for more than one MHC allele.

**Pentamer staining**

Phycoerythrin-conjugated Pro5 MHC Class I pentamers loaded with the immunodominant peptides listed in table 2 were used at 0.05 mg/ml, as suggested by the manufacturer (ProImmune). Frequencies of pentamer+ cells below 0.02% of CD3+ T cells were considered as background staining as indicated by the manufacturer.

A two-step staining at 4°C in the dark was performed. 1 x 106 PBMC were first incubated with PE-conjugated pentamers, washed once in PBS and then stained for surface markers. After two rounds of washing with PBS the cell suspensions were directly acquired on a Cytoflex flow cytometer (Beckman Coulter). All the antibodies were used at optimal dilutions (listed in the table below) as determined by preliminary titration experiments (not shown)

The table below contains information about antibodies used to characterize pentamer+ CD8 T cells

|  |  |  |  |
| --- | --- | --- | --- |
| **Antibody** | **Fluorochrome** | **Titer** | **Company** |
| anti-CD3 | APC-eFluor780 | 1:100 | eBioscience |
| anti-CD8 | PE-Cy7 | 1:100 | Beckman Coulter |
| anti-CD4 | ECD | 1:100 | Beckman Coulter |
| anti-CD45RA | ECD | 1:100 | Beckman Coulter |
| anti-CD279 (PD-1) | BV421 | 1:50 | Biolegend |
| anti-LAMP-1 (CD107a) | BV421 | 1:50 | Biolegend |
| anti-KLRG1 | FITC | 1:30 | Miltenyi Biotec |
| anti-CD127 | APC | 1:50 | Miltenyi Biotec |
| anti-IFNγ | APC | 1:50 | Becton Dickinson |
| anti-CD127 | BV785 | 1:30 | Biolegend |

**In vitro functional assays**

The ProMix EBV Peptide Pool (ProImmune), was added to PBMC cultured in RPMI 1640 (with 10% FCS and antibiotics/antimycotics) at the final concentration of 5 g/ml. Brefeldin A, Monensin and anti-human CD107a/LAMP-1 antibodies were added. In vitro stimulation was performed at 37°C and lasted 18 hours, after which cells were harvested and processed for surface and intracellular staining.

SPICE (Simplified Presentation of Incredibly Complex Evaluations) software was used for boolean analysis of EBV-reactive CD8 T cells in functional assays: each IFN+ cell was assigned to one of the 8 boolean subsets generated by the combination of positivity for KLRG1, CD127 and CD45RA, and the frequency of each subpopulation was measured.

**B cell characterization**

For the phenotypic characterization of B lymphocytes, prior to staining, 1x106 PBMCs were incubated for 15 minutes at 4° C in running buffer (PBS + 1% FBS + 0.5% EDTA 500mM) supplemented with FcR Blocking Reagent (Miltenyi Biotec) to reduce unspecific staining. Next, the fluorochrome conjugated monoclonal antibodies listed in the table below were added, and samples were incubated for 30 minutes at 4 °C in the dark, washed, and acquired on a Cytoflex flow cytometer.

|  |  |  |  |
| --- | --- | --- | --- |
| **Antibody** | **Fluorochrome** | **Titer** | **Company** |
| anti-CD24 | FITC | 1:30 | Becton Dickinson |
| anti-CD274 (PDL-1) | PE | 1:100 | BioLegend |
| anti-CD20 | ECD | 1:80 | Beckman Coulter |
| anti-CD38 | PerCP-Cy5.5 | 1:60 | Beckman Coulter |
| anti-IgD | PE-Cy7 | 1:160 | Pharmingen |
| anti-CD95 (FAS) | BV 421 | 1:30 | Biolegend |
| anti-CD69 | BV 421 | 1:60 | Pharmingen |
| anti-CD27 | BV786 | 1:30 | Becton Dickinson |
| anti-CD19 | APC-eFluor780 | 1:100 | eBioscience |
| anti-CD25 | PE | 1:60 | Becton Dickinson |

**Flow cytometry data analysis**

For each sample, approximately 300,000 lymphocytes were selected based on scatter parameters, and the analysis was conducted after the exclusion of dead cells (LIVE/DEAD Fixable Aqua Dead Cell Stain, ThermoFisher) and coincident events. Surface density of selected receptors was expressed in arbitrary median fluorescence intensity (MFI) units. The data was compensated and analyzed using FlowJo v10.5 (BD).