SUPPLEMENTAL MATERIAL

Ankylosing spondylitis patients present a distinct CD8 T-cell subset with osteogenic and cytotoxic potential

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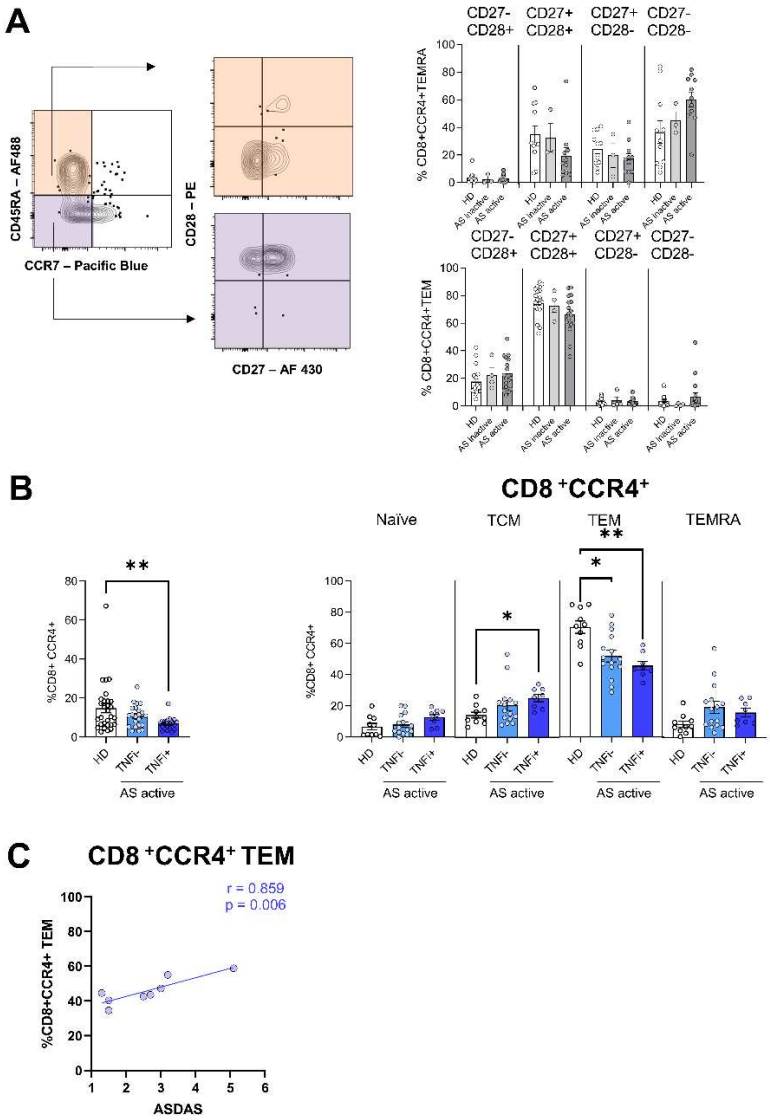
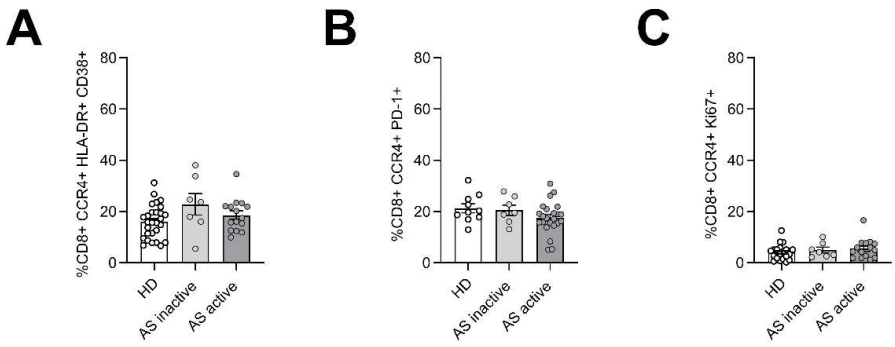
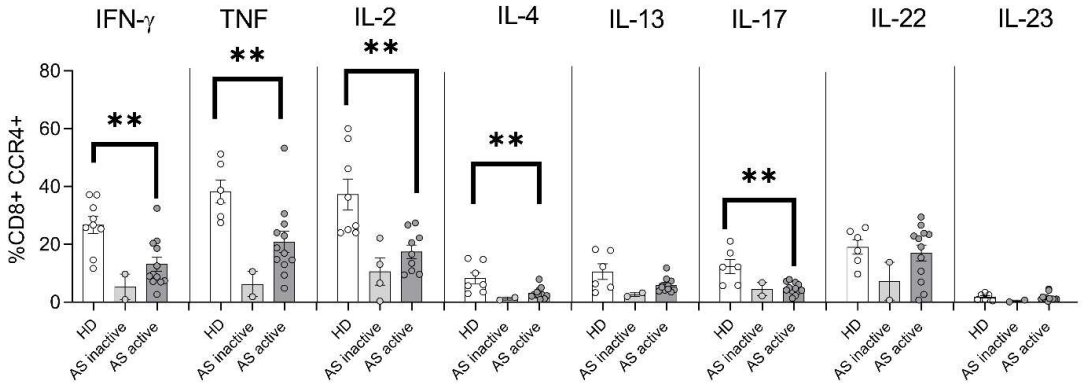


Supplemental Figure 1. Differentiation state and effect of TNF treatment on phenotype of CD8⁺CCR4⁺ T-cells. (A) Left: Identification of T_{EM} (purple) and T_{EMRA} (orange) based on the expression of CD45RA and CCR7 and subsequent analysis of CD27 and CD28 expression in these populations. Right: mean frequencies ± SEM of the expression of CD27 and CD28 by T_{EMRA} (top) and T_{EM} (bottom) in CD8⁺CCR4⁺ T-cells. (**B**) Left: Mean frequencies ± SEM of CD8⁺ T-cells expressing CCR4 in HD and patients with active AS under treatment with TNF inhibitors (TNFi+) or not treated with TNFi (TNF-). Right panel: memory phenotype frequencies of CD8⁺CCR4⁺ T-cells in HD and patients with active AS according to TNFi treatment. **(C)** Correlation between frequencies of CD8⁺CCR4⁺ T-cells with a T_{EM} phenotype and ASDAS in patients with active AS under TNFi treatment. Kruskal-Wallis was used to compare frequencies and phenotypes amongst the groups, asterisks indicate significant differences between the groups (* p<0.05, ** p<0.01).



Supplemental Figure 2. Activation and proliferation state of CD8⁺CCR4⁺ T-cells. Mean frequencies ± SEM of HLA-DR⁺ CD38⁺ **(A)**, PD-1⁺ **(B)** and Ki67⁺ cells **(C)** in CD8⁺CCR4⁺ T-cells.



Supplemental Figure 3. Cytokine secretion profile in CD8⁺CCR4⁺ T-cells. $CD8^+CCR4^+$ T-cells were sorted and stimulated with PMA and ionomycin for 5 hours. Intracellular cytokine staining was performed and mean frequencies of indicated cytokine secreting cells assessed by flow cytometry. Each symbol represents an individual and Kruskal-Wallis was used to compare markers between the groups, asterisks indicate significant differences between the groups (** $p<0.01$).

Supplemental Table 1. List of antibodies used for flow cytometry

Targeted Human Antigen	Fluorochrome	Clone	Isotype	Source
CCR4	PE-Cy7	L291H4	Mouse IgG1	BioLegend
CCR5	FITC	45523	Mouse IgG2b	R&D Systems
CCR6	PE	11A9	Mouse IgG1	BD Biosciences
CCR7	PE-Cy7 / Brilliant Violet 421	G043H7	Mouse IgG2a	Biolegend
CD27	V500	M-T271	Mouse IgG1	BD Biosciences
CD28	PE	CD28.2	Mouse IgG1	BioLegend
CD3	APC-Cy7	SP34-2	Mouse IgG1	BD Biosciences
CD38	Brilliant Violet 421	HB-7	Mouse IgG1	BioLegend
CD4	PerCP-Cy5.5	L200	Mouse IgG1	BD Biosciences
CD45RA	FITC	ALB11	Mouse IgG1	Beckman Coulter
CD8	APC	B9.11	Mouse IgG1	Beckman Coulter
CD8	BV786	RPA-T8	Mouse IgG1	BD Biosciences
CD8a	Pacific Blue	HIT8a	Mouse IgG1	BioLegend
CLA1	Brilliant Violet 421	HECA-452	Rat IgM	BD Biosciences
CX3CR1	PE	2A9-1	Rat IgG2b	BioLegend
CXCR3	Alexa Fluor 488 / PE	1C6/CXCR3	Mouse IgG1	BD Biosciences
Granzyme B	Pacific Blue	GB11	Mouse IgG1	BioLegend
HLA-DR	V500	G46-6	Mouse IgG2a	BD Biosciences
IFNγ	FITC	B27	Mouse IgG1	BD Biosciences
IL-2	PerCP-eFluor 710	MQ1-17H12	Rat IgG2a	eBioscience
IL-4	PE	MP4-25D2	Rat IgG1	BD Biosciences
IL-13	Brilliant Violet 421	JES10-5A2	Rat IgG1	BioLegend
IL-17A	Brilliant Violet 605	BL168	Mouse IgG1	BioLegend
IL-22	PerCP-eFluor™ 710	IL22JOP	Rat IgG2a	eBioscience
IL-23	eFluor™ 660	fc23cpg	Rat IgG1	eBioscience
Integrin β7	PE	FIB504	Rat IgG2a	BioLegend
Ki-67	Alexa Fluor 488	Ki-67	Mouse IgG1	BioLegend
PD-1	Brilliant Violet 785	EH12.2H7	Mouse IgG1	BioLegend
Perforin	APC	B-D48	Mouse IgG1	BioLegend
TNFα	Brilliant Violet 650	Mab11	Mouse IgG1	BioLegend

SUPPLEMENTAL METHODS

RNA sequencing

Library preparation and sequencing. The quality of the isolated RNA was determined with a Qubit (1.0) Fluorometer (Life Technologies) and a Fragment Analyzer (Agilent). Only those samples with a 260nm/280nm ratio between 1.8–2.1 and a 28S/18S ratio within 1.5-2 were further processed. NEBNext Ultra Directional RNA Library Prep for Illumina (New England BioLabs Inc.) and the NEBNext Multiplex Oligos for Illumina (New England BioLabs Inc.) kits were then used for library and cDNA synthesis and addition of barcode sequences, as per manufacturer's instruction. The sequencing of the libraries was performed in two batches using the NextSeq 500 (Illumina) with the NextSeq 500/550 High Output Kit v2.5 (75 cycles; Illumina) or with Novaseq 6000 (Illumina). Samples were processed starting from stranded, single-ended 75 bp-long sequencing reads. The sequencing was performed either at the Genomic Facility, Institute for Oncology Research or at the Functional Genomic Centre Zurich.

Bioinformatics analysis. RNA sequencing reads were aligned with the STAR-aligner [1]. The Ensembl human genome build GRCh38 was used as reference. Gene expression counts were computed with the function featureCounts from the R package Rsubread [2]. Gene expression analysis was performed using the BigOmics platform [3] (version v2.8.0), after batch correction made with the SVA method [4]. Differential gene expression analysis was computed by the platform using both edgeR and DEseq2 packages [5 6]. Gene set enrichment analysis was then performed on the obtained gene expression profile for each of the following comparison: active AS vs HD; active AS vs inactive AS; active ASe vs HD using the gene set variation analysis (GSVA) statistical method [7]. Gene Ontology Biological Process (GOBP) was used as a database for the gene set enrichment analysis, FDR<0.05 and | (log(Fold Change)) | >0.2 were set as statistical parameters. Using this approach, we could identify 153 differentially expressed GOBP in the comparison active vs HD (146 upregulated and 7 downregulated); 126 in the comparison active AS vs inactive AS (all upregulated) and 144 in the comparison inactive AS vs HD (1 upregulated and 143 downregulated).

SUPPLEMENTAL REFERENCES

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