SUPPLEMENTARY METHODS

Subject Recruitment

Clinical information was recorded for all patients, including demographic information (gender, age, smoking status and BMI), disease duration, *HLA-B27* carriage, sulfasalazine and TNFi treatment information, the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (1) and Bath Ankylosing Spondylitis Functional Index (BASFI) (1), and clinical manifestations (inflammatory back pain, uveitis, axial arthritis, peripheral arthritis, ulcerative colitis, Crohns disease, enthesitis, dactylitis and psoriasis). Dietary habits were assessed by a 52-question questionnaire to exclude subjects with special dietary habits such as an entirely plant-based or meat-based diet. A summary of the cohort characteristics is available in Supplementary Table 1.

Shotgun metagenome sequencing

Faecal samples were collected and stored at −80°C prior to DNA extraction. DNA was extracted using a StoolGen DNA kit (CWBiotech Co., Beijing, China). DNA concentrations were determined using a Qubit dsDNA BR assay kit (Thermo Fisher, Foster City, CA, USA). 200 – 500 bp insert size libraries were constructed using a TruSeq DNA Sample Preparation Kit (Illumina, San Diego, CA, USA) and an automated SPRI-Works system (Beckman Coulter, San Jose, CA, USA), Quality Control (QC) of each library was carried out using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), Qubit dsDNA BR assay kit (Thermo Fisher, Foster City, CA, USA) and a KAPA qPCR MasterMix plus Primer Premix kit (Kapa Biosystems, Woburn, MA, USA) according to the manufacturer's instructions. Libraries that passed QC (>3 ng/μL) were sequenced.

Microbiome Sequence Analysis

Abundance tables were arcsine square root transformed prior to analysis. Multidimensional data visualisation was conducted using a sparse partial least squares discriminant analysis (sPLSDA) as implemented in R as part of the MixOmics package v6.3.1 (5), at the species level using Bray-Curtis

distance matrices. Receiver operating characteristic curve was calculated using sPLSDA and cross-validation of the combined discovery and validation cohorts using the MixOmics package v6.3.1. Multivariate association of the bacterial species composition with sources of covariation was investigated using a PERMANOVA test (10000 permutations) as part of vegan v2.4-5 (6). Gender, BMI, age and smoking status were found to exhibit significant associations and were subsequently included as covariates in the downstream multivariate and univariate statistical models. Multivariate association of bacterial species composition with AS status and TNFi treatment was conducted using PERMANOVA (10000 permutations). Univariate association of bacterial species and functional pathways/groups were tested for significance using MaAsLin v0.0.5, using default parameters which includes Benjamini-Hochberg correction (7), and Wilcoxon rank-sum tests as implemented in R (8). Only results which were significant in both tests ($P < 0.05$) were reported in the main text. The alpha diversity of bacterial species was calculated using the rarefy function, as implemented in vegan v2.4-5. For measurement of microbial epitope richness, the Shannon, Simpson and Chao diversity indices were measured (vegan v2.4-5) and group differences were evaluated using Wilcoxon rank-sum tests. Genetic-relatedness dendrograms for strain-level results from PanPhlAn were calculated using Jaccard distance matrices and hierarchical clustering as implemented in R v.3.5.2.

T-cell responses to HLA-B27 presented bacterial peptides

Synthetic Peptides

All peptides were synthesized by SBSGene (Shanghai, China). The peptides were dissolved in DMSO for further use.

Study Subjects

Peripheral blood mononuclear cells (PBMCs) of AS patients and healthy donors were obtained from the Department of Rheumatology and Immunology of Shanghai Changzheng Hospital (Shanghai, China). The samples were stored in liquid nitrogen until use. All participants in the study have signed

an informed consent form. Ethical approval was obtained from the Ethics Committee of Shanghai Changzheng Hospital.

Generation of EBV-transformed B-LCLs

PBMCs (approximately 10^7 cells) separated from AS patients were suspended in 5ml RF-10 (RPMI-1640 supplemented with 10% FCS, 2mM L-glutamine, 5×10^{-5}M 2-ME, 100U/ml penicillin and 100 U/ml streptomycin, Gibco) with the presence of FK-506 (20nM, InvivoGen). Then 5ml supernatant of B95-8 cell culture was added into the cells. The cells were cultured in a 37°C, 5% CO2 incubator. The growth of B lymphoblastic clumps could be seen after 3-6 days transformation. Transformed B-LCLs were sent for HLA-B analysing and stored in liquid nitrogen for further using.

Generation of antigen-specific CD8+ T cells

For the primary stimulation, a total number of 10^7 PBMCs of an individual patient or healthy donor were separated into two parts to generate two T cell culture. In one culture, 4.5×10^6 of the cells (in 1ml RF-10) were put in 24-well tissue culture plate, from which 0.5×10^6 of the cells were divided into eight parts and each part was pulsed with a single peptide (10^{-6} mmol/L) in 200 µL RF-10 for 1 hour (peptides listed in Supplementary Table 9). These 8 parts were combined, washed with PBS, and then added into the original cells, and cultured in RF-10 with the presence of 20 IU/ml recombinant human interleukin-2 (rIL-2) (Peprotech, Brisbane, QLD, Australia).

Before re-stimulation, CD4+ T cells were depleted with magnetic beads (Dynabeats CD4, Invitrogen, USA). Autologous B cell lymphoblastoid cell lines (B-LCLs) were used as antigen presentation cells (APC) for re-stimulation. The B-LCLs (10 percent of the total cell number) were irradiated and divided into eight parts to pulse with a single peptide individually, then added to the culture plate as described above.

Assessing antigen-specific CD8+ T cell functions

Nine days after restimulation, an intracellular cytokine staining (ICS) was performed to assess antigen-specific CD8+ T cells. For identification of each peptide, 10^5 cells and a test peptide (10^{-6} mmol/L) were plated into U-bottom 96-well plate with 10^5 autologous B-LCLs added. Then, the cells were incubated in 37℃ and 5% CO2 for 5h with the presence of Brefeldin A (BFA) (Sigma) before ICS. Fluorescent anti-CD4, anti-CD8 and anti-IFN-γ antibodies (BD) were used to identify the specific CD8+ T cells.

Supplementary References

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