Figure S1. Anti–IFN-α autoantibodies block phosphoSTAT-1 and phosphoSTAT-4 production
Plasma from all patients was tested against plasma from normal donors with representative examples of 3 independent experiments shown below. (A) Normal PBMC incubated with 10% plasma from 1 normal, patient #10 (without anticytokine autoantibodies), and patients #8 and #9 (with anti–IFN-α autoantibodies). PBMCs were left unstimulated or stimulated for 15 minutes with IFN-α or IFN-γ. Cells were fixed and stained for phosphoSTAT-1. (B) Normal PBMC underwent PHA/IL-2 induced blastogenesis and incubation with plasma as above, and cells stimulated with either IFN-α or IL-12. Cells were fixed and stained for phosphoSTAT-4.

Figure S2. Impact of autoantibodies on phosphoSTAT-1 production and IFN-induced gene expression in vitro
(A) Flow cytometry using normal PBMC incubated with 10% plasma from, 1 normal, patient #13 with no anti–IFN-β autoantibodies, and patients #2 (with non-neutralizing anti–FN-β autoantibodies), and patients #8, #9, and #11 (with neutralizing anti–IFN-β autoantibodies). Cells were left unstimulated or stimulated with IFN-β or IFN-γ for 15 min and stained for phosphoSTAT-1. Representative examples of 3 independent experiments shown. (B) Immunoblot on lysates were prepared from PBMC incubated with normal or patient plasma and stimulated with IFN-α, IFN-β, or IFN-γ. Blots were probed for phosphoSTAT-1 and total STAT. β-actin was also used to ensure equivalent protein loading. A representative blot from four independent set of experiments is shown. (Ci and Cii) PBMC obtained from healthy donors (n=4) were cultured in the presence of normal and patient plasma (10%) (4 patients with anti–IFN-α autoantibodies and 3 patients with anti–IFN-β autoantibodies) and stimulated with IFN-α, IFN-β (1,000U/mL) or IFN-γ (400U/mL) for 3 hours; (Ciii and Civ) PBMC from a normal donor when treated with plasma from patient #2 with non-neutralizing anti–IFN-β autoantibodies. Target gene expression was evaluated by real time PCR and values are mean fold induction (± SD) relative to the non stimulated cells. GAPDH was used as normalization control.

Figure S3. Anti–IL-12 autoantibodies are biologically active in vitro
(A) Normal PBMC-derived lymphoblasts incubated with 10% plasma from 1 normal, patient #10 (no anti-cytokine autoantibodies) and patients #7 and #9 (with both IFN-α and IL-12p40 and p35 autoantibodies). Lymphoblasts were left unstimulated or stimulated with IFN-α or IL-12 for 15 minutes and stained for phosphoSTAT-4. Representative example of 3 independent experiments shown. (B) Normal PBMC in 10% normal or patient plasma were left unstimulated or stimulated with PHA plus IL-12 or IFN-γ plus LPS and measured for IL-12 or IFN-γ–induced IL-12 at 48 hours. Patients organized by which, if any autoantibody present by LIPS against IL-12 subunits p35 or p40. (C) Normal PBMC in 10% normal or patient plasma were left unstimulated or stimulated with PHA (1%) plus IL-12 (100ng/mL) and measured for IL-12–induced IFN-γ production at 48 hours.

Figure S4. anti–IL-1a and anti-IL17 autoantibodies are biologically active in vitro
(A) PBMC incubated in 10% normal plasma; normal plasma with commercial IL-1a antibody; or patient plasma with high-titer, low-titer or no anti–IL-1a autoantibodies and stimulated for 48 hours with PHA. Cell supernatants measured for IFN-g production. (B) HFF-1 cells in 10% normal plasma; normal plasma with commercial IL-1a antibody; or patient plasma with high-titer anti–IL-1a autoantibodies and stimulated with IL-1a for 8 hours. Cell lysates harvested for qRT-PCR of IL-1a responsive genes, CXCL11 and ICAM1. (C) HFF-1 cells incubated in 10% normal or patient plasma containing IL-17 autoantibodies and stimulated for 24 hours with IL-17. Supernatants collected for measurement of IL-17–induced IL-6 production.
B

Nonstimulated
PHA (1%) + IL-12 (1ng/mL)
IFN-g (1000U/mL) + LPS (200mg/mL)

C

Nonstimulated
PHA (1%) + IL-12 (1ng/mL)

Figure S3
Figure S4

A

IFN-γ (pg/mL)

NL IL-1α 1 7 8

Commercial High-titer Low-titer No IL-1α auto-ab

PHA

B

Relative fold change

NSIL-1α NSIL-1α NSIL-1α

Commercial High-titer (patient #1)

CXCL11 ICAM1

C

IL-6 (pg/mL)

No infections

Mucocutaneous candidiasis and other OI

Unstimulated IL-17 (100ng/mL)

Normal #1 #2 #13