ARTICLE - Bone Marrow Failure

Impaired immunosuppressive role of myeloid-derived suppressor cells in acquired aplastic anemia

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Received: November 2, 2021.
Accepted: June 13, 2022.
Prepublished: June 23, 2022.

https://doi.org/10.3324/haematol.2021.280292

Supplementary Materials and Methods

Characterization of cell phenotype

Cell staining was performed using fluorescein isothiocyanate (FITC) conjugated anti-Lin (CD3, CD14, CD16, CD19, CD20, CD56), phycoerythrin (PE) conjugated anti-HLA-DR, allophycocyanin (APC) conjugated anti-CD33, and phycoerythrin-cyano dyes 7 (PE-Cy7) conjugated anti-CD11b, peridinin chlorophyl II protein- cyano dyes 5.5 (PerCP-Cy5.5) conjugated anti-CD14, Brilliant Violet 510 (BV510) conjugated anti-CD15 mouse anti-human monoclonal antibodies (BD Biosciences Pharmingen, San Diego, CA, USA). To determine the expression level of Arg-1 and iNOS, cells were fixed and permeabilized using the Intracellular Fixation & Permeabilization Buffer Set (eBioscience, USA) according to the manufacturer's instructions. The monoclonal Brilliant Violet 421 (BV421) conjugated anti-Arg-1 and FITC conjugated anti-iNOS antibodies (Biolegend, San Diego, CA, USA) were incubated for 1h.

The phenotype of in vitro generated MDSCs was evaluated for the cell surface markers of CD33, CD11b, HLA-DR and CD14. Changes in PBMC subpopulations during cytokine induction and rapamycin modulation, as well as intracellular molecular of iNOS and Arg-1, were measured by flow cytometry.

Supplemental Table 1. Clinical characteristics of patients with AA

Baseline characteristic	
Gender	
Male, No. (%)	30(46.20%)
Female, No. (%)	35(53.80%)
Median age (years, range)	33(13-70)
Severity of disease, No. (%)	
NSAA	24(36.90%)
SAA	27(41.50%)
VSAA	14(21.50%)
Bone marrow hypoplasia, No. (%)	
<10%	29(44.60%)
10-20%	15(23.10%)
20-30%	6(9.20%)
30-40%	4(6.20%)
40-50%	7(10.80%)
NA	4(6.20%)
Cell counts (median, range)	
WBC ($\times 10^9$/L)	2.31(0.11-4.38)
ANC ($\times 10^9$/L)	0.64(0.00-2.35)
ARC ($\times 10^9$/L)	23.40(2.60-94.90)
PLT ($\times 10^9$/L)	18(2-80)
Hb (g/L)	72(33-137)

AA: aplastic anemia; NSAA: non-severe aplastic anemia; SAA: severe aplastic anemia; VSAA: very severe aplastic anemia; WBC: white blood cell; ANC: absolute neutrophile granulocyte; ARC: absolute reticulocyte; PLT: platelet; Hb: hemoglobin.

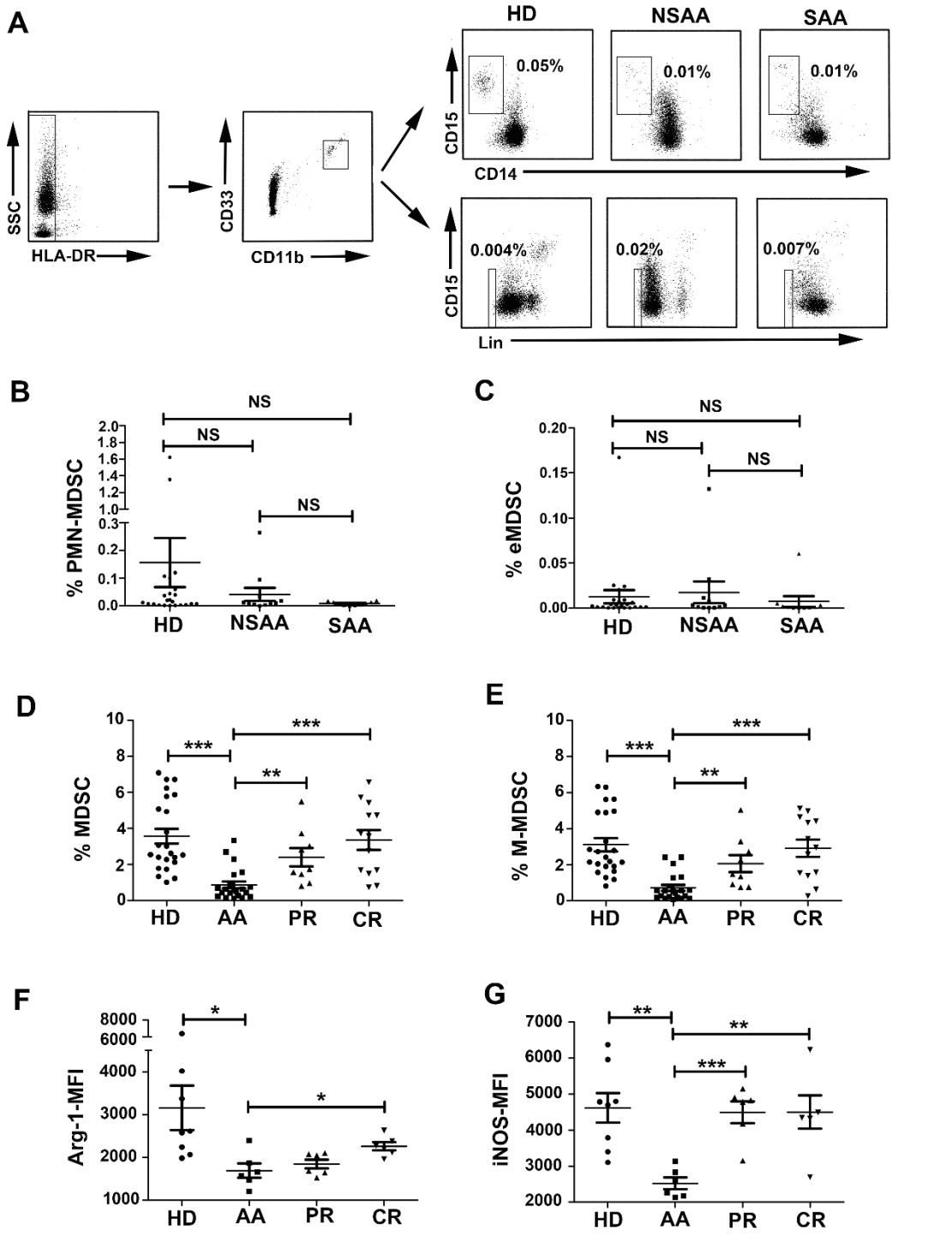
Supplemental Table 2. The number of MDSC subsets in the peripheral blood

	HD	NSAA	SAA
No.	23	11	7
MDSC			
mean±SEM (%)	3.57±1.99	1.11±1.10	0.67±0.76
P value		0.001[a]	<0.001[b]
M-MDSC			
mean±SEM (%)	3.11±1.75	0.87±0.87	0.58±0.68
P value		<0.001[c]	<0.001[d]
PMN-MDSC			
mean±SEM (%)	0.16±0.42	0.05±0.79	0.01±0.01
P value		0.415[e]	0.284[f]
eMDSC			
mean±SEM (%)	0.12±0.03	0.03±0.05	0.01±0.02
P value		0.276[g]	0.661[h]

HD: healthy donor; NSAA: non-severe aplastic anemia; SAA: severe aplastic anemia; MDSC: myeloid-derived suppressor cell; M-MDSC: monocytic-myeloid-derived suppressor cell; PMN-MDSC: polymorphonuclear-myeloid-derived suppressor cell; eMDSC: early-stage myeloid-derived suppressor cell. [a,b], The percentage of MDSC in NSAA (a) and SAA (b) patients were lower than HD; [c,d], The percentage of M-MDSC in NSAA (c) and SAA (d) patients were lower than HD; [e,f], The percentage of PMN-MDSC in NSAA (e) and SAA (f) patients was not distinguishable with HD;

[g,h], The percentage of eMDSC in NSAA (g) and SAA (h) patients was not distinguishable with HD.

Supplemental Figure 1

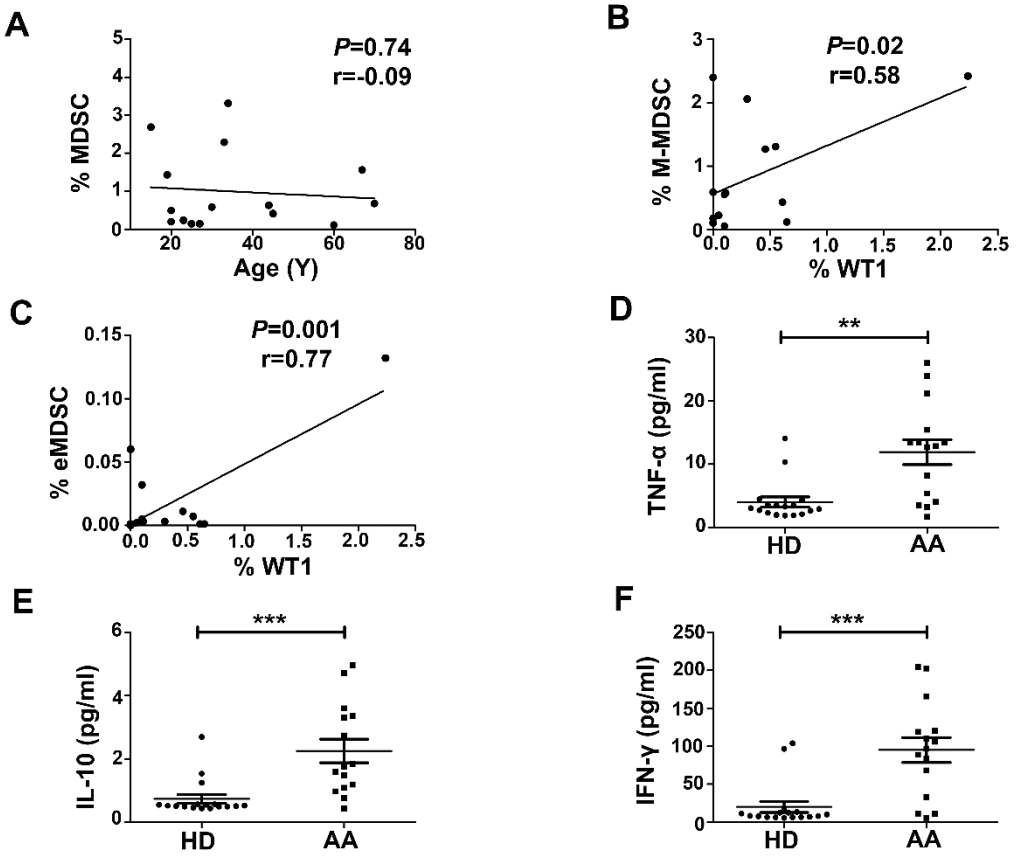


Supplemental Figure 1. Decreased number of myeloid-derived suppressor cells (MDSCs) in the peripheral blood of aplastic anemia (AA) patients.

A, The representative cytograms of MDSC subsets $CD33^{+}CD11b^{+}HLA\text{-}DR^{-}$ $CD15^{+}$ polymorphonuclear (PMN)-MDSCs and early-stage MDSCs (eMDSCs) within the gate of peripheral blood mononuclear cells (PBMNCs). B, C, The percentage of PMN-MDSCs (B) and eMDSCs (C) in PBMNCs from non-severe AA (NSAA) patients (n=11), severe AA

(SAA) patients (n=10) and healthy donors (HDs) (n=23). D, E, The percentage of MDSCs (D) and M-MDSCs (E) were elevated in AA patients with partial response (PR) (n=9) and complete response (CR) (n=13). F, G, The level of Arg-1 (F) and iNOS (G) were increased in AA patients with PR (n=6) and CR (n=6). NS, not significant; Lin, lineage-specific markers (Lin); HLA-DR, human leukocyte antigen-D-related; Arg-1, arginase-1; iNOS, inducible nitric-oxide synthase; *P<0.05, ** P<0.01, *** P<0.001.

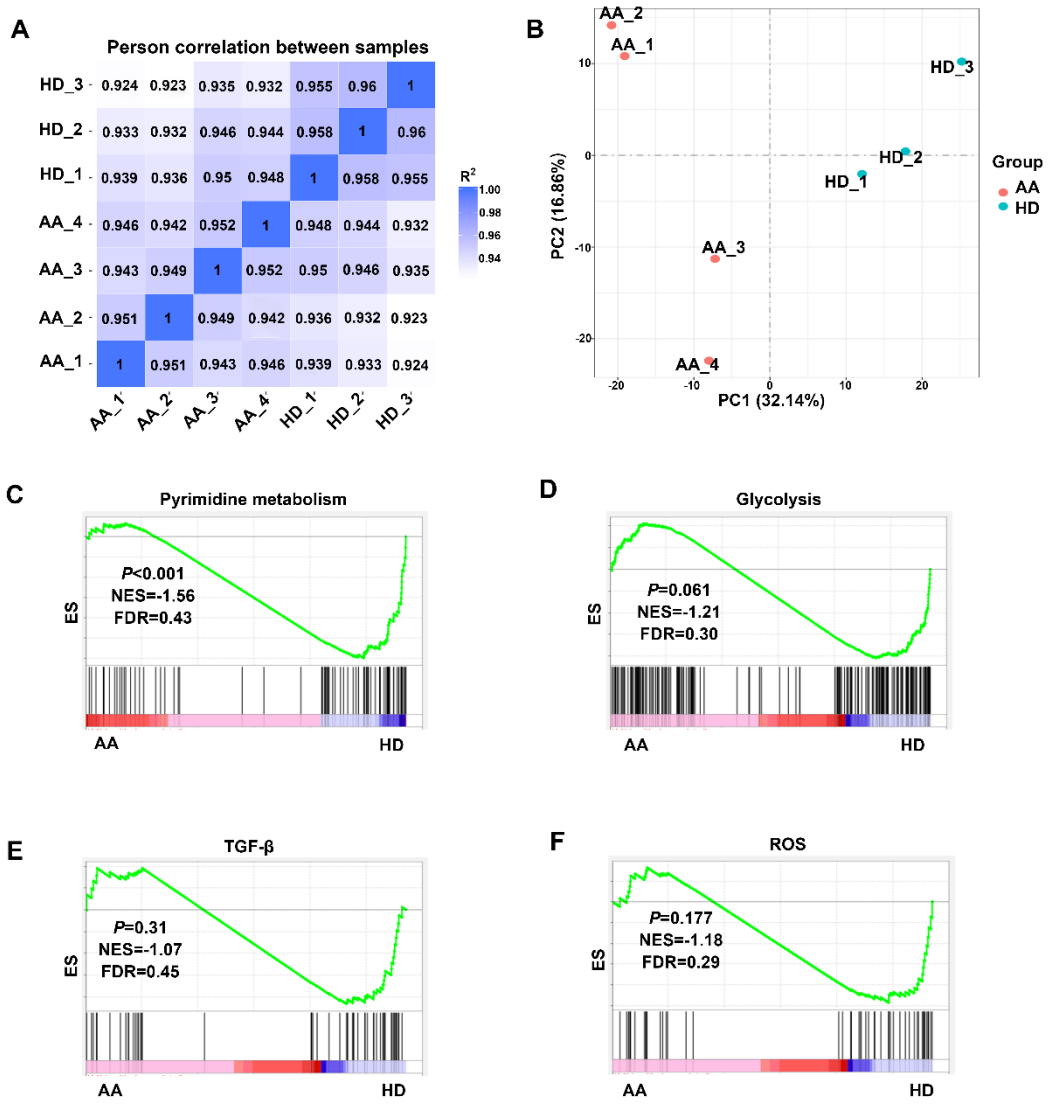
Supplemental Figure 2



Supplemental Figure 2. Relationship between myeloid-derived suppressor cells (MDSCs) and clinical characteristics of aplastic anemia (AA).

A, Relationship between MDSC and age (n=15). B, C, Percentages of monocytic (M)-MDSC and eMDSC were positively correlated with WT1 levels (n=15). D-E, Plasma levels of tumor necrosis factor (TNF)-α (D), IL-10 (E) and interferon (IFN)-γ (F) of AA (n=15) and healthy donors (HD) (n=17) determined by cytometric bead array.

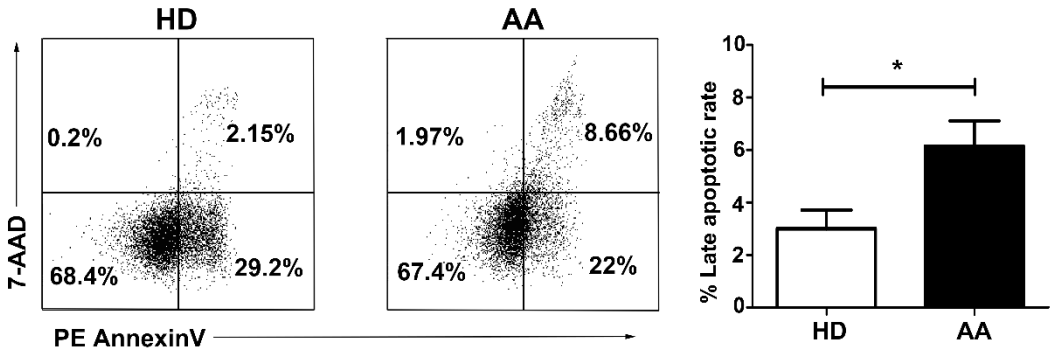
Supplemental Figure 3



Supplemental Figure 3. The gene expression pattern of myeloid-derived suppressor cells (MDSCs) from aplastic anemia (AA) patients and healthy donors (HDs).

A, Correlation analysis between samples. B, A clear clustering between AA and HD MDSCs was observed by principal component analysis. C-F, Gene set enrichment analysis reveals pyrimidine metabolism (C), glycolysis (D), transforming growth factor (TGF)-β (E), reactive oxygen species (ROS) pathways in AA MDSCs compared with HD MDSCs. ES, enrichment score.

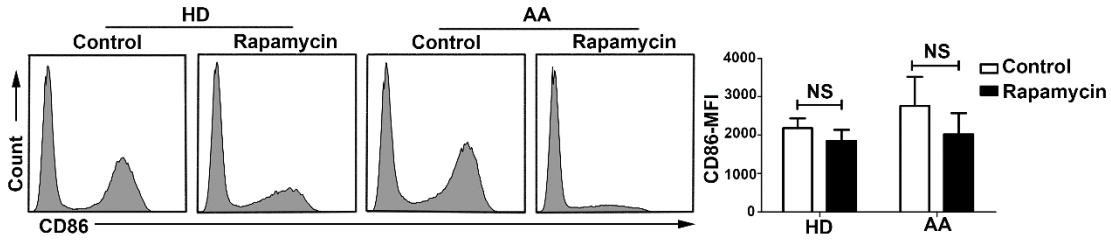
Supplemental Figure 4



Supplemental Figure 4. The apoptotic rates of MDSCs from patients with AA and HD were detected by flow cytometry.

Peripheral blood mononuclear cells were stained with CD14, HLA-DR, 7-AAD and Annexin V. Compared with HD, significantly increased late apoptotic cells (7-AAD$^+$ Annexin$^+$) were detected in MDSCs (CD14$^+$ HLA-DR$^-$) from AA patients (HD: n=5; AA: n=5). AA, aplastic anemia; HD, healthy donor; *P<0.05.

Supplemental Figure 5



Supplemental Figure 4. The expression of CD86 from patients with AA and HD was detected by flow cytometry (HD: n=5; AA: n=4).

Peripheral blood mononuclear cells were treated with rapamycin or control for 6 days before lipopolysaccharide (LPS; 1μg/ml) stimulation for 24 hours. All quantitative data represent mean±SEM. NS, not significant; AA, aplastic anemia; HD, healthy donor.