CD8+ T cell exhaustion in sarcoidosis disease

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Introduction

Sarcoidosis is a unique disease which is at the crossroads of microbial infection and host immune response. Specifically, sarcoidosis is a multisystem granulomatous disease of unknown etiology, characterized by a Th1 immunophenotype. Recent published work from our lab demonstrates antigen-specific recognition of mycobacterial proteins in CD4+ T cells derived from 72% of sarcoidosis subjects at diagnostic bronchoscopy. Furthermore, 60% of sarcoidosis subjects exhibited antigen-specific recognition by CD8+ T cells. The unexpected finding of antigen-specific CD8+ T cells suggests that CD8+ T cells may play an important role in sarcoidosis pathogenesis. Accordingly, CD8+ T cells have been well studied in chronic infection. These studies demonstrate that persistent antigen stimulation leads to antigen-specific CD8+ T cell exhaustion, characterized by upregulation of inhibitory receptors such as PD-1 that negatively regulate antigen-specific cells. Therefore we hypothesized that T cell exhaustion contributes to sarcoidosis disease pathogenesis.

Methods

Ten sarcoidosis and five healthy control subjects were enrolled in this study (Table 1: subject demographic detail). Subject PBMC were stimulated polyclonal T cell stimulation using plate bound anti-CD3 and soluble anti-CD28 antibodies. PD-1 expression was then measured on CD8+ T cells by multiparameter flow cytometry. Concurrently, intracellular and extracellular IFN-γ and IL-2 production were assessed by cytokine bead array.

Table 1. Demographic characteristics of the study population

Characteristics	Sarcoidosis	Healthy Subjects
Number	10	5
Sex, female/male	6/4	3/2
Age (yr), median (min, max)	40 (28,64)	45 (26,61)
Race[1]	6AA:4C	3AA:2C
CD4/CD8 ratio, median (min,max)	4.96 (3.15,26.7)	1.80 (1.26,2.1)

Results

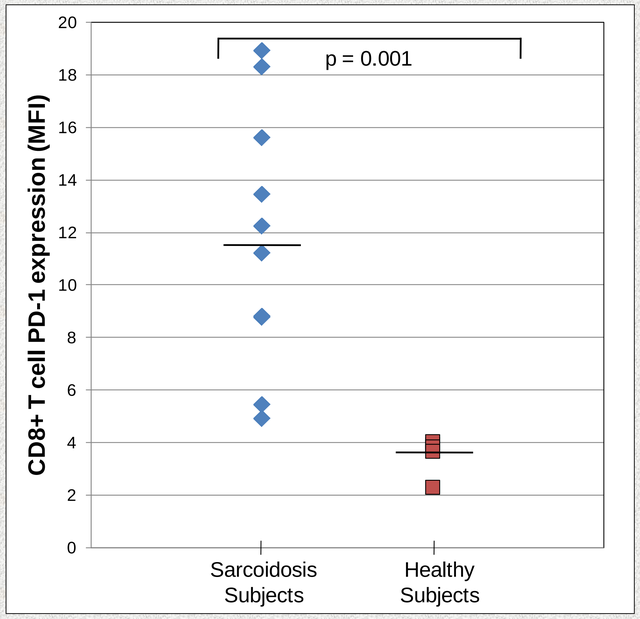

Figure 1: Sarcoidosis CD8+ T cells display increased PD-1 expression. PBMC were activated using plate bound anti-CD3 and soluble anti-CD28 antibodies. PD-1 expression was then measured by multiparameter flow cytometry at day 3 post-activation. Sarcoidosis CD8+ T cells expressed increased PD-1 as compared to healthy subjects (p = 0.001).

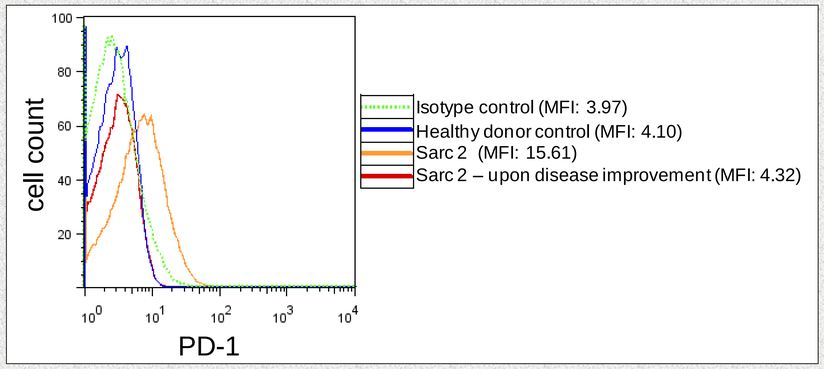

Figure 2: Representative flow cytometry analysis plot for CD8+ T cell PD-1 expression. CD8+ T cells were stained with PD-1 or isotype control. Healthy subjects display low PD-1 expression. PD-1 expression returns to healthy subjects levels in sarcoidosis patient with disease improvement. Improvement measured by decreased diameter/resolution of skin granulomas and improved lung function.

	IFN-γ (pg/mL)		IL-2 (pg/mL)		PD-1[1]	
Subject	Pre	Post	Pre	Post	Pre	Post
SARC 1	56356	95346	3383	4708	13.45	15.01
SARC 2	33676	9332	4751	1258	15.61	4.32
SARC 3	14910	1997	10766	1288	18.93	15.88
SARC 4	197003	123873	3549	4038	5.43	3.56
SARC 5	63985	60171	1566	1201	8.76	6.95
SARC 6	4073	26865	197	694	18.31	4.39

[1]CD8+ T cell PD-1 expression

Figure 3. Disease improvement in sarcoidosis subjects results in decreased PD-1 expression. PBMC were obtained from subjects during active disease and post-disease improvement (sarcoidosis subjects 2-6). Sarcoidosis subject 1 did experience disease improvement; both time points subject maintained active disease. PBMC were activated using plate bound anti-CD3 and soluble anti-CD28 antibodies. Supernatants were collected at 24 hours post-activation and analyzed for IFN-γ and IL-2 by cytometric bead array. PD-1 expression was measured at day 3 post-activation.

Results

- PD-1 expression was upregulated on CD8+ T cells in sarcoidosis subjects as compared to healthy subjects.
- In five of the sarcoidosis subjects, we assessed PD-1 expression during active disease and upon disease improvement. Improvement of disease correlated with reduction in PD-1 expression.
- In four of the five sarcoidosis subjects, disease improvement resulted in reduced inflammatory cytokine production.

Conclusions

- These results indicate that CD8+ T cell exhaustion, as measured by PD-1 expression, may correlate with sarcoidosis disease activity.
- Reduction in inflammatory cytokines may contribute to CD8+ T cell recovery from exhaustion.
- Furthermore, these data suggest that the manifestations of chronic infection may contribute to sarcoidosis disease pathogenesis.

Acknowledgements

This work was supported by the ATS/Foundation for Sarcoidosis Research grant to K.R. as well as the NIH grants: K01-HL10317901 to K.R. and RO1-HL83839; R01-AI65744; MO1 RR-00095; Vanderbilt CTSA grant 1 UL1 RR024975 to W.P.D.