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1 **Th17/regulatory T cells balance is predictive of *Coccidioides* infection outcome in pediatric**
2 **patients**

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15
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22 **FOOTNOTES**

23 *Conflict of interest*. The authors have nothing to disclose.

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31

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36 **ABSTRACT**

37 Background: Protective immunity against the fungal pathogen *Coccidioides* requires specific T
38 helper responses. Mouse vaccine and infection studies have defined $CD4^+$ T helper (Th)1 and
39 Th17 cells in the resolution of infection and in effective protection. Patients with persistent
40 *Coccidioides* infection demonstrate reduced cellular responses.

41 Methods: Peripheral blood and serum were collected from 30 pediatric *Coccidioides*-infected
42 patients and 20 healthy controls in the California San Joaquin Valley. Samples were evaluated by
43 flow cytometry for innate and adaptive immune populations and cytokines to define the early
44 immune response and identify clinically useful biomarkers for predicting disease outcome.
45 Clinical and flow data were evaluated according to disease outcome (resolved or persistent)
46 using principal component analysis, high-dimensional flow cytometry analysis tools, chi-square
47 automatic interaction detection, and individual cell population comparisons.

48 Results: Patients with persistent infection had lower Th17 and higher Treg frequencies, but
49 similar Th1 responses, relative to patients that resolved disease. Treg frequency, eosinophil
50 numbers and neutrophil numbers together distinguish patients that resolve infection from those
51 that develop persistent infection.

52 Conclusions: The inability to resolve *Coccidioides* infection may be a result of elevated Treg
53 frequency and functional capacity, and Treg frequency may predict patient disease outcome at
54 diagnosis. In our study, Th1 responses were similar in persistent and resolved infection, in
55 contrast to prior human studies. Instead, our data suggest that Th17 cells provide an effective
56 protection during *Coccidioides* infection, and that elevated Treg frequency inhibits protective
57 immunity.

58

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59 **INTRODUCTION**

60 Coccidioidomycosis, also known as Valley fever, is caused by a fungal pathogen endemic to the
61 San Joaquin Valley in California, Arizona, northern Mexico and arid areas of South America [1].
62 Population expansion, travel, improved detection and/or climate changes appear to be
63 contributing to an expanding endemic region. 40% of those infected develop symptomatic
64 pneumonia that typically resolves without anti-fungal therapy, although disease can become
65 chronic and disseminate outside the lungs [1]. Persistent coccidioidomycosis is thought to be due
66 to poor or ineffective cellular immunity.
67
68 Cellular immune responses are critical for effective immunity to *Coccidioides* infection [2, 3].
69 Early mouse studies indicate a need for T helper 1 (Th1) responses in protective immunity [4].
70 More recent studies highlight a role for Th17 cells and IL-17 cytokine responses [5-9]. Effective
71 Th1 responses have been linked to resolution of human *Coccidioides* infection [10, 11]; however,
72 Th17 effectors are critical for clearance of many other fungal pathogens.
73
74 Tregs function to control immune responses during infection and disease [12]. Th17 and Treg
75 differentiation are inversely regulated, with IL-6 promoting TGFβ-induction of Th17 cells and
76 inhibiting TGFβ-induction of Tregs [13-15]. An appropriate balance in T effector and regulatory
77 cells allows effective immune responses while limiting tissue damage. Very little is known about
78 the role of Tregs in the regulation of the immune response during *Coccidioides* infection. In mice
79 infected with *Coccidioides posadasii*, Treg expansion correlated with reduced survival [16]. In
80 other fungal infections, Treg expansion correlates with impaired T cell immunity and persistent
81 disease [17-19]. IL-10, an anti-inflammatory cytokine produced by several adaptive immune

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82 cells including Tregs, promotes survival of *Coccidioides* and is associated with less-effective
83 immunity [20, 21]. Tregs secrete IL-10 as one cellular immune suppression mechanism.
84

85 METHODS

86 *Study enrollment and design*

87 Patients were enrolled and samples collected at Valley Children's Healthcare (VCH), a 355 bed
88 Children's Hospital serving as tertiary referral center for 10 counties in central California. Thirty
89 children aged 2-18 years with coccidioidomycosis diagnosed by positive coccidioidal serology or
90 cultures demonstrating *Coccidioides species* were included. Of these, 15 were enrolled as
91 inpatients and 15 as outpatients at the time of diagnosis. Twenty healthy siblings of hospital
92 patients, with negative coccidioidal serology were enrolled as controls. Children known to be
93 pregnant, immunocompromised and/or on immunosuppressive medications, with severe
94 underlying illness, cystic fibrosis, or inflammatory diseases were excluded. The study was
95 approved by the VCH institutional review board. Written informed consent was obtained from
96 legal guardians and participants >7 years of age. Baseline demographic, clinical, laboratory,
97 radiographic, antifungal treatment and outcome data were collected. Outcomes were defined as
98 resolved or persistent infection at the time of study conclusion. Resolution of symptoms,
99 abnormal radiographic and clinical laboratory findings defined disease resolution. Persistent
100 disease was defined as still on therapy without complete resolution of symptoms and/or
101 persistent abnormal radiographic or laboratory findings.
102

103 Patients were characterized by 42 clinical parameters (Supplemental Table 1), 51 immune cell
104 population parameters (Supplemental Table 2), and 26 serum proteins. Frequency and total

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105 number per milliliter of innate and adaptive immune populations were evaluated in the peripheral
106 blood at the time of enrollment. We compared percentages, total number and activation state of
107 immune cells in patients based on disease outcome (healthy control, resolved, persistent) and
108 hospital status at time of blood draw (inpatient, outpatient).
109
110 *Serum and peripheral blood collection.*
111 1ml whole blood collected in sodium heparin tubes was stored at room temperature, and
112 processed within 24-hours for flow cytometry. 1ml collected in red-cap plug tubes was
113 centrifuged at 3500rpm for 15min then transferred into new tubes for –20°C storage.
114
115 *PBMC flow cytometric analysis.*
116 Antibody staining was performed in PBS/2%FBS. 100µL of whole blood was incubated with
117 5µL of Human TruStain FcX (Biolegend) for 5min prior to staining. Three flow cytometry
118 panels were designed to profile PBMCs (Supplemental methods). A single blood sample was
119 processed three times such that 50µL of antibody for each panel was added and incubated for
120 20min. RBCs were lysed in 1-step Fix/Lyse Solution (eBioscience) for 25min and resuspended
121 with 300µL PBS/2%FBS and 100µL of 123count-eBeads (eBioscience) for flow cytometry
122 analysis. Stained PBMC were acquired on an LSRII (BD), and analyzed using FCS Express
123 (DeNovo Software) FACS Diva (BD).
124
125 To ensure consistent cytometer calibration and data collection, voltage and compensation
126 parameters were standardized using SPHERO Ultra Rainbow Calibration Particle Kits
127 (Spherotech, Inc.). Consistent MFI for each fluorophore was maintained by small adjustments to

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128 voltage parameters if baseline MFI changes were >10% between experiments. Fluorophore
129 compensation was maintained with antibody single stains mixed from 1µL of each antibody into
130 UltraComp eBeads (Bioscience), washed with PBS/2%FBS, incubated for 15min, and
131 resuspended in 500µL PBS/2%FBS.
132
133 *High-dimensional flow cytometry analysis.*
134 FCS files were loaded into Cytobank (www.cytobank.org), and pre-gated to remove counting
135 beads, debris, doublets, and dead cells. Remaining cells were gated on CD4+CD45+, grouped on
136 outcome, and analyzed using CITRUS and viSNE. File internal compensation was utilized,
137 clusters characterized on abundance, event sampling equalized per file, minimum cluster size set
138 to 0.5%, and Significance Analysis of Microarrays (SAM) correlative model utilized. CITRUS
139 analysis was repeated three independent times to confirm stratifying signatures and statistically
140 significant populations (discovery rate <1%). viSNE map was generated from 373,781 total
141 events (12,889/patient) using 2500 iterations, a perplexity of 90, and a Theta of 0.3.
142
143 *Cytokine assay.*
144 Thawed serum was centrifuged at 1000x*g* for 5min to remove particulates. Serum cytokine
145 concentrations were determined using LEGENDplex Human 13-plex kits, ‘Th Cytokine Panel’
146 and ‘Cytokine Panel 2’ bead-based immunoassay kits (Biolegend) in duplicate according to the
147 manufacturer’s instructions. Samples were diluted 2-fold with kit Assay Buffer prior to assay
148 initiation. Samples were analyzed by flow cytometery and processed using LEGENDplex Data
149 Analysis Software. Standard curves were generated using a 5-parameter curve-fitting model and
150 cytokine levels were calculated as the average of the duplicate measurements.

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151

152 *Data analysis and statistics.*

153 Leukocyte subset total numbers were calculated using counting beads in combination with each
154 leukocyte percentage determined by flow cytometry. Statistical analyses were performed using
155 Prism software v6.0 (GraphPad Software). One-way ANOVA with Bonferroni correction was
156 used to compare multiple groups with a 95% confidence interval. The Fisher's exact test was
157 used to compare the distribution of groups and percentages. PCA and CHAID analyses were
158 performed using XLSTAT.

159

160 **RESULTS**:

161 **Patient characteristics**

162 Thirty pediatric patients with a diagnosis of coccidioidomycosis (15 inpatients and 15
163 outpatients) and 20 healthy controls were enrolled. One inpatient died due to disease severity and
164 was excluded from analysis. Of the thirty patients with coccidioidomycosis, 25 had pulmonary
165 involvement and five had disseminated disease including the deceased patient. Resolved disease
166 was observed in 13 (44.8%) patients and persistent disease in 16 (55.2%), including 12 with
167 pulmonary and 4 with disseminated disease.

168

169 Age and ethnicity, as well as the incidence of chest pain, night sweats, weight loss and fatigue
170 symptoms were similar between the groups, and did not correlate with disease outcome (Table 1
171 and Table 2). More females than male patients were enrolled in the study, but this is unlikely to
172 be due to an increased incidence in female children [22, 23]. Regardless of disease severity or
173 outcome, most patients experienced fever. As observed previously, erythema nodosum mildly

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174 correlated with a better disease prognosis (p=0.0722). Evaluating complete blood count (CBC)
175 measurements, inpatients had higher neutrophil, eosinophil, WBC and platelet numbers, and
176 lower lymphocyte numbers compared to outpatients (Supplemental Figure 1). Eosinophil,
177 platelet and creatinine (Cr) levels were elevated and lymphocyte counts reduced in patients with
178 persistent disease compared to patients that resolved infection. Although some CBC parameters
179 were significantly different between the groups, there was considerable overlap in values and
180 these small variations would be difficult to use in predicting disease outcome. Serum IgM, IgG
181 and antibody titers from *Coccidioides* enzyme immunoassay and complement fixation (CF)
182 assays did not correlate with disease outcome, and were not different between inpatient and
183 outpatients (Supplemental Table 3).
184
185 Analysis of immune parameters based on hospital status revealed several differences between
186 inpatients and outpatients in both innate and adaptive responses. Plasmacytoid dendritic cell
187 frequency was reduced in inpatients relative to healthy controls and outpatients (Figure 1A).
188 $CD4^+$, $CD8^+$ and B cell frequency was reduced in inpatients, and mild elevation of Th1 was
189 observed (Figure 1B, C). These differences likely reflect the more severe illness, elevated
190 inflammatory responses and ineffective adaptive and Th cell immunity of the inpatients. A larger
191 proportion of inpatients versus outpatients was unable to resolve *Coccidioides* infection, and
192 included the four patients with disseminated disease, suggesting the more severe disease at
193 diagnosis, the more likely that disease will persist.
194
195 **Specific adaptive immune responses distinguish disease outcome**

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196 No differences were observed in the frequency or total number of innate immune populations
197 based on disease outcome (data not shown). Patients with persistent disease tended to have lower
198 adaptive immune cell frequency, in particular a significant reduction in B cell frequency relative
199 to healthy controls and resolved patients (Figure 2A). Persistent patients also had fewer Tfh cells,
200 important for effective and diverse antibody responses. Current paradigm suggests that a strong
201 Th1 response is required for controlling *Coccidioides* infection. Patients with IFNγR or Stat1
202 mutations that reduce Th1 responses have more severe, disseminated infection [24]. Th1
203 frequencies were similar between patients with resolved and persistent disease (Figure 2B),
204 while patients with persistent disease had lower Th17 and higher Treg frequencies than patients
205 that resolved disease.
206
207 Immune cells secrete inflammatory and effector cytokines to induce cell migration,
208 differentiation and function. We evaluated the concentration of 26 inflammatory and Th
209 cytokines (Figure 3A). As cytokines were evaluated in serum, and not from stimulated cell
210 supernatants, most cytokines were expressed at just above the limit of detection (LOD). IL-1α,
211 IL-1β, IL-5, IL-12p70, IL-21 and IL-22 were below the LOD for all patients (not shown).
212 Significant differences in IL-6, IL-18 and IL-12, and mild difference in IL-10, were observed
213 between patients with resolved and persistent disease, and all of these were increased relative to
214 healthy controls (Figure 3B). IL-6, IL-18 and IL-12 are produced by antigen presenting cells and
215 promote T effector differentiation. IL-10, an immunosuppressive cytokine, is expressed by
216 several immune populations, including Tregs. IL-18 inhibits Th17 and promotes Treg
217 differentiation and function [25, 26].
218

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219 **Predictive biomarkers of disease outcome identified**

220 To identify predictive biomarkers we performed principle component analysis (PCA) using all
221 non-diagnostic clinical, immune population and cytokine parameters. PCA analysis separated
222 patients from healthy controls, and inpatients from outpatients, but was unable to distinguish
223 patients with resolved versus persistent disease (Figure 4A). PCA comparison, using all
224 parameters, only described 31.9% of variation in the hospital status data and 26.3% of variation
225 in the disease outcome data, indicating that some parameters are too variable to include as a
226 means to distinguish patients. Chi-square automatic interaction detection (CHAID), a decision
227 tree technique, was used to define a predictive method for disease outcome. Treg frequency had
228 the greatest impact on disease outcome of all clinical and immune parameters (Figure 4B). Treg
229 frequency at diagnosis distinguished disease outcome in 79.3% of patients (11/13 resolved and
230 12/16 persistent disease). Eosinophil, neutrophil and Th17 numbers further defined patients
231 within these disease outcomes (not shown). PCA using the parameters identified by CHAID in
232 the first two levels of separation (%CD4^{+}Treg, eosinophils and neutrophils), predicted disease
233 outcome in 89.7% (26/29) of patients.
234

235 **Unbiased high-dimensional flow cytometry analysis reveals CCR5 as a biomarker of**
236 **persistent disease**

237 We applied high-dimensional flow cytometry analysis tools to identify cellular populations that
238 predict disease outcome. We utilized CITRUS, a tool that identifies stratifying cellular signatures
239 that differ between grouped data [27]. After pre-gating CD4^{+}CD45^{+} T cells, we utilized CD127
240 (IL-7Rα), CXCR5 (CD185), CCR3 (CD193), CCR5 (CD195), CCR6 (CD196), CD25 (IL-2Rα),
241 and HLA-DR to cluster the data. Comparing resolved versus persistent disease patients, CITRUS

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242 identified several cell populations that stratified the data at a 1% false discovery rate, including
243 one population representing Tregs (Figure 5A). CITRUS clusters revealed CCR5 (CD195) as
244 expressed more highly in persistent patients; specifically, elevated CCR5 stratified Tregs. The
245 frequency of the CCR5+Treg population was higher in persistent than resolved disease (Figure
246 5B). CCR5 is critical for Treg migration into tissues [28].
247
248 Performing viSNE, a dimensionality reduction and data visualization tool [29], we confirmed a
249 region of Tregs that could be divided on CCR5 expression. (Figure 5C) [29, 30]. CCR5^{+}Tregs
250 were significantly elevated in patients with persistent disease as compared to patients who
251 resolved infection, while CCR5^{neg}Treg frequency was unchanged (Figure 5D). High-dimensional
252 analysis confirms a Treg disparity based on disease outcome, and reveals CCR5 as a functional
253 marker associated with chronicity.
254

255 **DISCUSSION**

256 We identified several clinical measurements and immune populations that together provide
257 potential biomarkers for predicting disease outcome in *Coccidioides*-infected patients. Elevated
258 Treg frequency was the major indicator of persistent infection. Most patients with persistent
259 disease remain on anti-fungal treatments for months to years, with risk of fungal reactivation
260 upon therapy discontinuation. Understanding host immune responses leading to dissemination,
261 persistent infection or resolution of infection may allow clinicians to identify patients with
262 persistent infection earlier.
263

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264 Patients with persistent disease appear to develop an inappropriately prolonged innate immune
265 response, and ineffective adaptive immune activation to *Coccidioides* infection. Expanded
266 neutrophils and eosinophils indicate that the innate immune system in these patients mounts an
267 inflammatory response, in contrast to reduced lymphocyte frequencies. As lymphocytes and
268 appropriate $CD4^+$ T cell responses are required for immune control of *Coccidioides*, patients
269 with persistent infection likely do not have sufficient activated lymphocyte numbers to control
270 infection. Resolution of infection appears to require a Th17, but not a Th1, response that is
271 reduced in patients with persistent infection.

272

273 The four patients who developed disseminated infection had significantly reduced alanine
274 transaminase (ALT) levels compared to resolved patients (p=0.0014; not shown), indicating liver
275 dysfunction not previously described in disseminated coccidioidomycosis, although elevated
276 aspartate aminotransferase:ALT ratio is observed in disseminated histoplasmosis [31]. We
277 observed trends indicating reduced adaptive immune responses (reduced $CD4^+$, $CD8^+$, B, and
278 Th17 cells) and ineffective innate immune responses (elevated classical monocytes) [32]. Further
279 study of disseminated infection is needed to clarify the immune responses in disseminated
280 coccidioidomycosis.

281

282 Tregs suppress T cell activation and effector mechanisms [30]. Enhanced Treg ratios and
283 suppressive function may block appropriate adaptive immune responses, enabling prolonged
284 fungal infection, resulting in persistent infection. Elevated Treg frequency provided the best
285 biomarker for identifying these patients at diagnosis, suggesting that Tregs may suppress
286 appropriate immune responses during *Coccidioides* infection. Alternatively, as Tregs and Th17

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287 cells differentiate through reciprocal pathways, Tregs may be generated at the expense of Th17
288 cells required for fungal clearance, resulting in persistent infection [15]. These patients may have
289 naturally high Treg frequencies before infection, or as suggested by the significant elevation in
290 Treg frequency relative to healthy controls, may ineffectively induce peripheral Tregs instead of
291 Th17 effectors. Serum cytokine levels in patients with persistent infection further support the
292 possibility of inappropriate T cell differentiation. IL-18 inhibits Th17 and enhances Treg
293 differentiation and function, which could explain persistent infection in these patients [25, 26].
294
295 In *Paracocidioides brasiliensis* infection Treg frequency and suppressive function is enhanced
296 relative to controls [18]. Our results support a similar finding in *Coccidioides* infection, with
297 higher Treg frequencies indicative of persistent infection, and markers of suppressive Treg
298 function. CITRUS analysis revealed a higher $CCR5^{+}$Treg frequency in persistent infection.
299 $CCR5^{+}$Tregs have enhanced suppressive capacity compared to $CCR5^{neg}$Tregs [33]. CCR5-
300 deficient mice have an enhanced ability to combat *Paracoccidioides brasiliensis* infection, and
301 elevated Th17 response to *Histoplasma* infection, resulting in a Treg/Th17 imbalance [34, 35].
302 We speculate that Treg expansion and elevated functionality prevent appropriate Th17 effector
303 responses allowing persistent *Coccidioides* infection.
304
305 The elevated Treg frequency and potential suppressive functionality is consistent with our
306 understanding of Tregs in infection, and with the inverse relationship in Th17 and Treg
307 differentiation. It is tempting to propose that manipulating Tregs or Th17 cells could improve
308 prognosis for *Coccidioides*-infected patients. Further predictive modeling is needed to confirm
309 our findings, and better understand the Treg phenotype during infection. To our knowledge, this

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310 is the first pediatric study demonstrating higher Treg and lower Th17 levels in patients who
311 cannot clear infection. These markers could identify patients earlier in their disease course who
312 would benefit from prolonged treatment course, and close monitoring for disease relapse once
313 off therapy.
314

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325

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FIGURE LEGENDS

Figure 1. Immune parameters based on patient hospital status. PBMCs from healthy and *Coccidioides*-infected patients were assessed by flow cytometry. A) Comparisons of monocytes and dendritic cell frequencies. Frequency comparisons of adaptive immune populations $CD4^+$, $CD8^+$ and B cells (B), and of $CD4^+$ T helper subsets as a percentage of $CD4^+$ T cells (C). Each dot represents an individual patient, and lines indicate the mean. ANOVA with Bonferroni correction was used to compare multiple groups with a 95% confidence interval; * $p<0.05$, ** $p<0.005$, *** $p<0.0005$.

Figure 2. T helper cells determine disease outcome. PBMCs from acute disease were incubated with antibodies and the percentage of adaptive immune populations (A) and T helpers (B) determined by flow cytometry. T helper subset frequencies are represented as percentage positive within the $CD4^+CD45^+$ gate. Samples were separated by healthy, resolved or persistent infection. Long lines indicate the mean, and each dot represents an individual patient. ANOVA with Bonferroni correction was used to compare multiple groups with a 95% confidence interval; * $p<0.05$, ** $p<0.005$, *** $p<0.0005$.

Figure 3. Serum cytokine data. Serum was incubated with a panel of cytokine antibodies and the concentration determined by flow cytometric analysis. Samples were separated by healthy, resolved or persistent infection. A) Cytokines are shown with the mean and SEM. IL-1α, IL-1β, IL-5, IL-12p70, IL-17A, IL-21, and IL-22 were below the LOD, and are not shown. B) Individual cytokines are shown with each dot representing an individual patient. Samples at or

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348 below the LOD are shown at the LOD. ANOVA, then a Fisher's Least Significant Difference
349 was performed; * $p<0.05$, ** $p<0.005$.
350
351 **Figure 4. PCA and CHAID analysis reveals a relationship between Treg frequency and**
352 ***Coccidioides* infection.** A) PCA using 119 clinical and flow cytometry parameters of controls,
353 inpatients and outpatients (left graph), or controls, resolved and persistent disease (right graph).
354 B) CHAID analysis on persistent and resolved patients identifies a relationship between %CD4$^+$
355 Treg and disease outcome. C) PCA analysis on persistent and resolved patients using eosinophil
356 and neutrophil numbers, and %CD4$^+$ Treg parameters.
357
358 **Figure 5: CCR5$^+$ Tregs are a significant predictor of disease outcome.** CITRUS analysis was
359 performed comparing persistent and resolved patients. Within the CD4$^+$CD45$^+$ population,
360 CITRUS identified several differential cell clusters that separated patient groups. CD127, CCR5,
361 and CD25 expression is shown for the Treg cluster (A). Box plots display the frequencies of the
362 significant Treg cluster between persistent versus resolved patients demonstrating this cluster is
363 elevated in patients who go on to have persistent infection (B). (C) viSNE heatmaps were
364 generated for CD25, CD127, and CCR5 on two representative patients. Gates were drawn around
365 two Treg (CD4$^+$CD45$^+$CD127^{low}CD25$^+$) clusters based on CCR5 expression. %CCR5$^+$ Tregs
366 and %CCR5^{neg} Tregs was determined across all samples (D). An unpaired student's t-test was
367 performed.
368
369

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Figure 1. Davini et al.

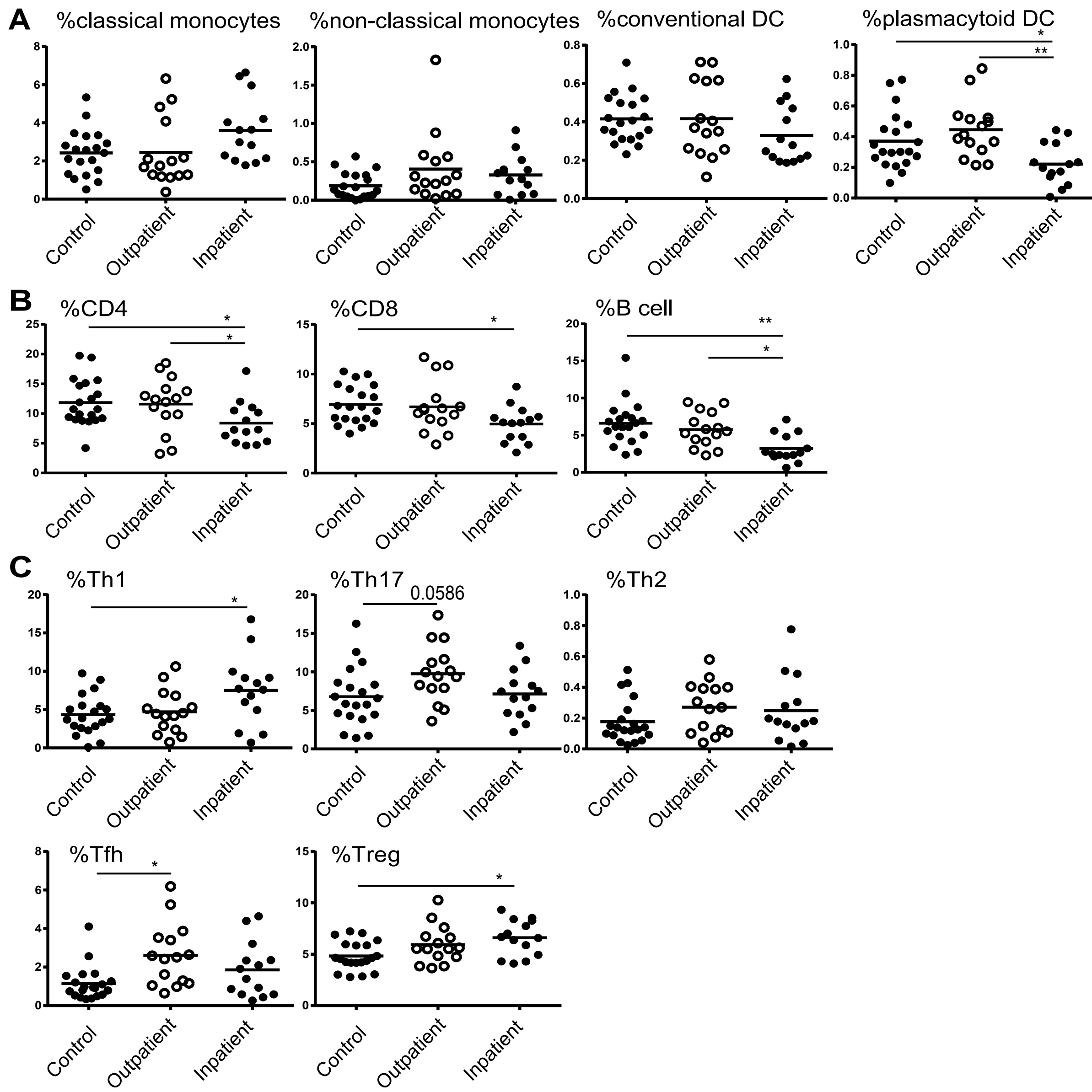

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Figure 2. Davini et al.

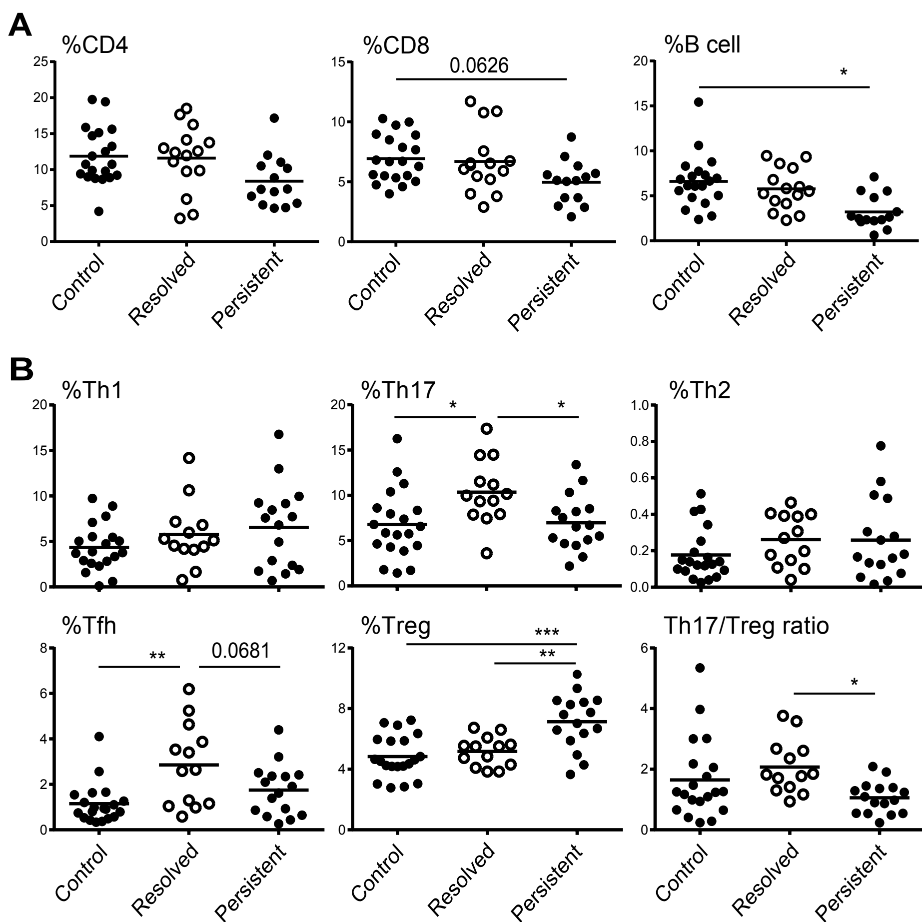

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Figure 3. Davini et al.

A

pg/ml

0 5 10 15 20

IL-2 IL-4 IL-6 IL-9 IL-10 IL-13 IL-17F TNFa TSLP GMCSF IFNa IL-23

0 200 400 600

IFNg IL-12p40 IL-15 IL-18 IL-11 IL-27 IL-33

Control
Resolved
Persistent

B

IL-6

pg/ml

1 10 100

** *

Control Resolved Persistent

IL-10

1 10 100

* 0.0581

Control Resolved Persistent

IL-12p40

1 10 100 1000

* *

Control Resolved Persistent

IL-18

10 100 1000 10000

** *

Control Resolved Persistent

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Figure 4. Davini et al.

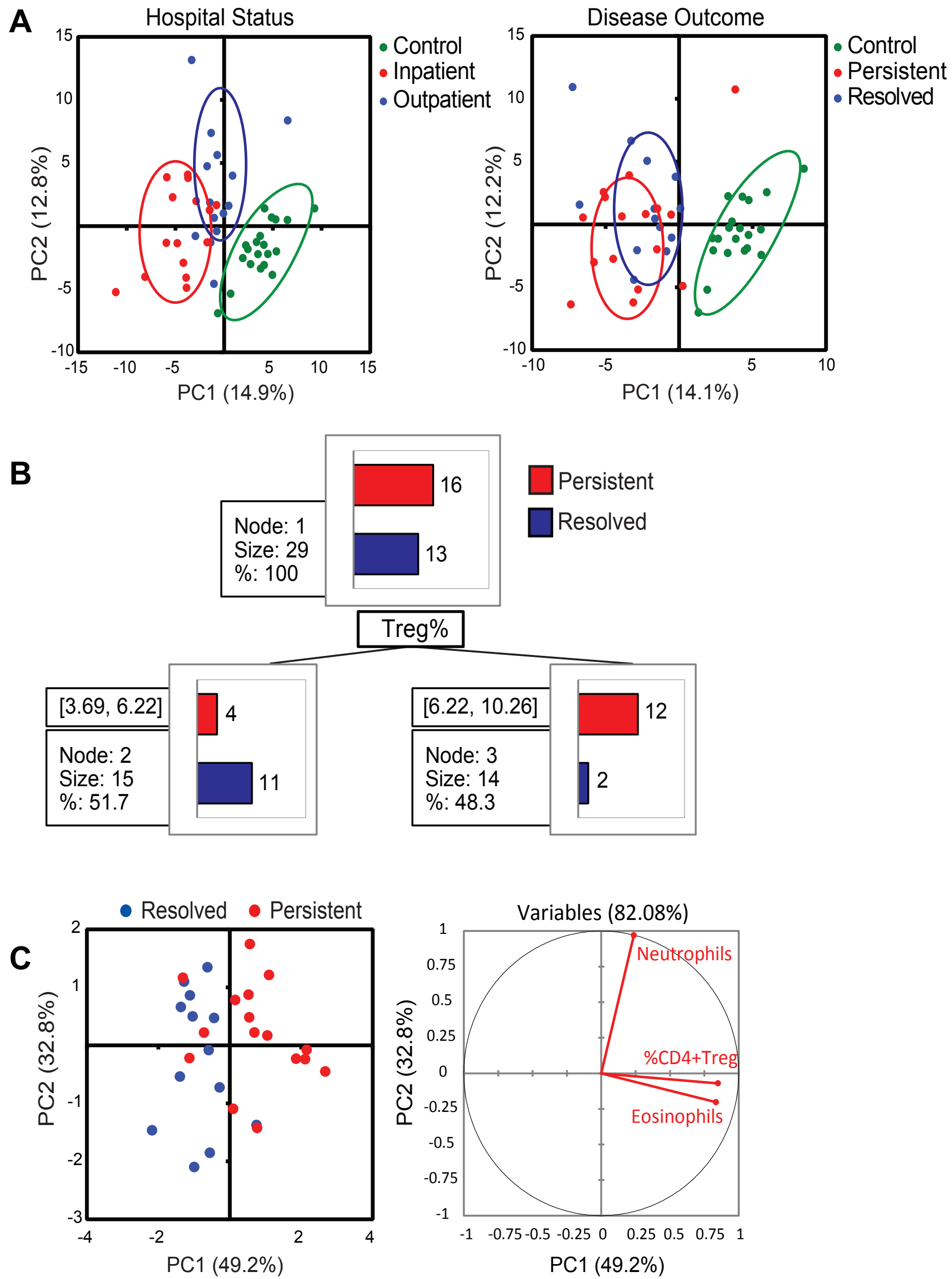

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Figure 5. Davini et al.

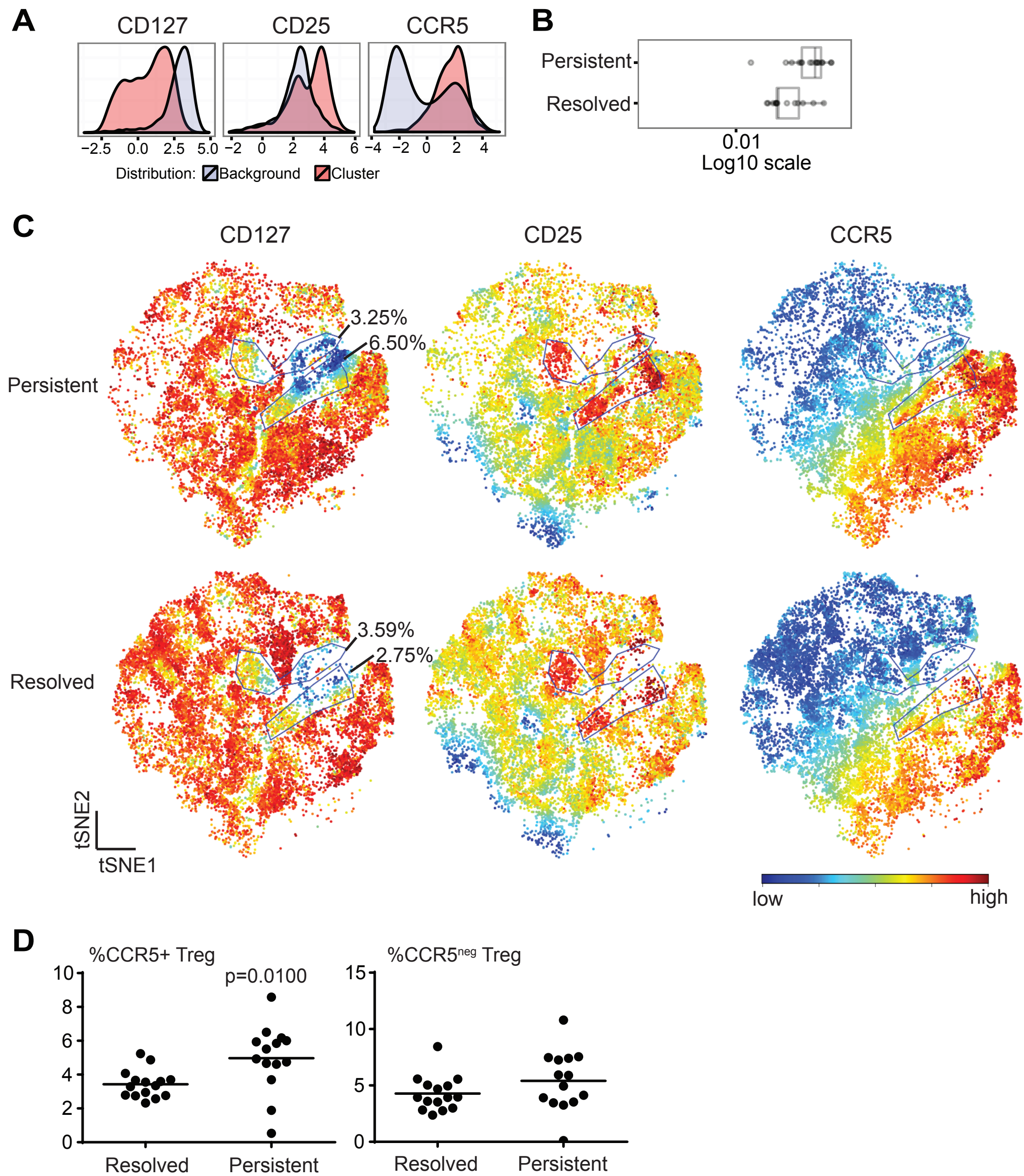

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Table 1. Demographics of patients based on outcome status.

	Healthy Controls (n=20)	Resolved (n=13)	Persistent (n=16)
Age (median, range), yrs	11, 3-16	12, 6-18	13, 2-17
Gender, n (%)			
Male	11 (54%)	3 (23.1%)	5 (31.3%)
Female	9 (45%)	10 (76.9%)	11 (68.8%)
Ethnicity, n (%)			
Hispanics	14 (70%)	11 (84.6%)	14 (87.5%)
Non Hispanics	6 (30%)	2 (15.4%)	2 (12.5%)
Hospitalization status, n (%)			
Inpatients	0	2 (15.4%)	12 (75.0%)
Outpatients	20 (100%)	11 (84.6%)	4 (25.0%)

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Table 2. Clinical features of pediatric *Coccidioides*-infected patients.

	Resolved (n=13)	Persistent (n=16)
Fever, n (%)	11 (94.6%)	13 (18.8%)
Cough, n (%)	7 (53.8%)	10 (62.5%)
Chest pains, n (%)	5 (38.5%)	8 (50.0%)
Night sweats, n (%)	3 (23.1%)	5 (31.3%)
Fatigue, n (%)	4 (30.8%)	5 (31.3%)
Weight loss, n (%)	1 (7.7%)	1 (6.3%)
Erythema nodosum, n (%)	7 (53.8%)	3 (18.8%)
# of clinical parameters per patient (avg +/- stdev)	2.9 +/-1.2	2.8+/-1.4
# Medications per patient, n (%)		
0	1 (7.7%)	0
1	12 (92.3%)	9 (56.3%)
2	0	4 (25.0%)
3	0	3 (18.8%)
First medication, n (%)		
Fluconazole	12 (92.3%)	11 (68.6%)
Ambisome	0	5 (31.3%)
X-ray, n (%)		
Infiltrates	6 (46.2%)	9 (56.3%)
Consolidation	4 (30.8%)	10 (62.5%)
Adenopathy	1 (7.7%)	4 (25.0%)

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Not tested	0	2 (12.5%)