

METHODS

Information about prior skin testing was available in 36 asthmatic patients, and 27 of the 36 patients had positive skin test results to inhaled allergens. Comorbid conditions in the 40 asthmatic patients included obesity (16 patients), hypertension (15 patients), reflux esophagitis (11 patients), dyslipidemia (9 patients), obstructive sleep apnea (6 patients), hypothyroidism (5 patients), and diabetes mellitus (4 patients). In the asthmatic patients there was no difference in total numbers of CD45⁺Col1⁺, differentiated CD45⁺Col1⁺α-SMA⁺, or activated CD45⁺Col1⁺Smad2/3⁺ fibrocytes between patients with and without each of these comorbid conditions. Similarly, regression analysis did not disclose any significant relationship between age and circulating total, differentiated, or activated fibrocyte numbers.

Asthma medications and absence of effect on fibrocytes

Although 13 of the 15 patients in the GINA^{low} group were using ICSs at the time of the study, all of the patients in the GINA^{high} group were

taking ICSs. Regression analysis was performed to assess an effect on fibrocytes, and there was no significant relationship between daily ICS dose and fibrocyte subset levels. An effect of either OCSs or leukotriene receptor antagonists was examined in patients being treated and medication-naïve patients, and no demonstrable effect was evident on circulating total, differentiated, or activated fibrocyte numbers for either medication.

REFERENCES

- E1. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999;159:179-87.
- E2. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012;40:1324-43.

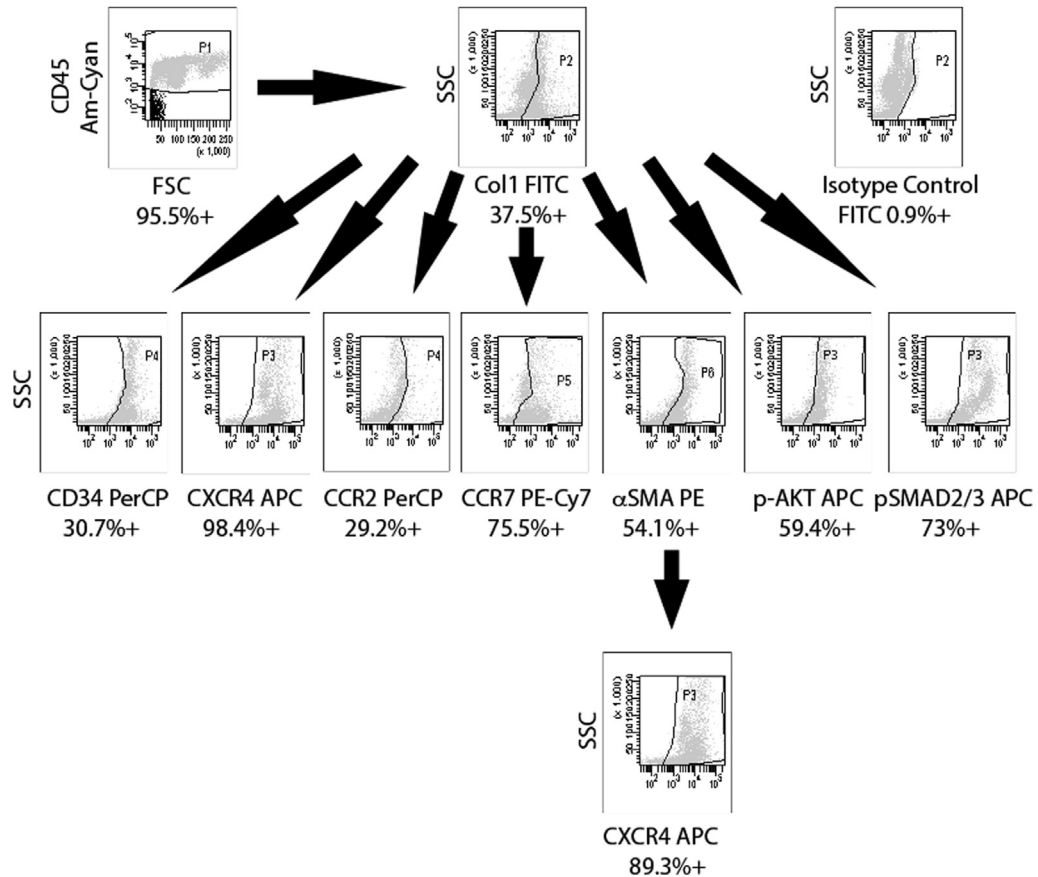


FIG E1. Flow cytometric gating for quantitation of total fibrocytes and fibrocyte subsets. Gating was performed on total leukocytes from the buffy coat fraction of peripheral blood samples to identify CD45⁺ (Am-Cyan [P1]) cells that were Col1⁺ (fluorescein isothiocyanate [P2]). Subsequent panels were gated on P2 (CD45⁺Col1⁺ cells). Arrows depict multicolor staining strategies to identify expression by the CD45⁺Col1⁺ cells of CD34 (PerCP); CXCR4 (APC), CCR2 (PerCP), and CCR7 (PE-Cy7); α -SMA (PE) and CXCR4 (APC); p-AKT (APC); and p-Smad 2/3 (APC). Single staining was used for compensation to eliminate bleed-over between fluorochromes. Isotype controls for all antibodies were used to set negative gates. Fluorescence minus one was used to compensate for shifts caused by multiple fluorochrome staining. The percentage on the *horizontal axis* represents the percentage of cells from the previous gate that were positive for the stain. *FSC*, Forward scatter; *SSC*, side scatter.

TABLE E1. Characteristics of asthmatic patients

Age (y)	Sex	BMI (kg/m ²)	GINA score	Before bronchodilator		After bronchodilator	IgE (IU/mL)	Severe exacerbation
				FVC (L [% predicted])	FEV ₁ (L [% predicted])	FEV ₁ (L [% predicted])		
43	F	29	4	2.9 (90%)	2.1 (78%)*	2.1 (81%)	10	
44	M	25	4	3.6 (72%)*	2.4 (63%)*	4.0 (105%)	478	Y
64	M	40	5	2.6 (63%)*	1.0 (32%)*	1.3 (43%)*	226	
79	M	31	4	3.5 (74%)*	1.8 (51%)*	1.9 (56%)*		
61	F	29	5	2.8 (79%)*	1.6 (60%)*	1.8 (66%)*	141	Y
40	F	51	5	3.0 (72%)*	2.1 (64%)*	2.5 (75%)*	36	
70	F	27	3	1.7 (57%)*	1.2 (52%)*	1.5 (69%)*	11	
75	F	30	4	1.3 (45%)*	0.6 (30%)*	0.8 (39%)*		Y
39	M	34	4	3.1 (63%)*	1.6 (40%)*	2.3 (57%)*	192	
63	F	27	4	2.4 (73%)*	1.4 (56%)*	1.6 (65%)*	99	Y
71	F	30	2	2.6 (91%)	1.8 (82%)	2.2 (101%)	83	Y
49	F	36	4	2.2 (69%)*	1.4 (56%)*	1.8 (72%)*	64	Y
23	M	37	5	3.2 (73%)*	2.4 (66%)*	2.8 (74%)*		Y
61	F	27	4	3.4 (84%)*	2.3 (72%)*	2.5 (79%)*	11	
65	M	40	5	3.5 (66%)*	2.3 (58%)*	2.8 (69%)*	1840	Y
58	M	31	5	2.8 (66%)*	1.8 (54%)*	2.1 (66%)*	40	Y
63	F	29	5	1.3 (42%)*	0.7 (29%)*	1.2 (47%)*	57	Y
53	F	33	4	3.2 (99%)	2.3 (88%)	2.8 (104%)	185	Y
57	F	32	5	1.3 (49%)*	1.0 (45%)*	0.9 (43%)*	14	Y
61	M	28	1	4.6 (97%)	3.4 (95%)	3.8 (101%)	41	
25	M	24	5	3.3 (60%)*	1.5 (33%)*	1.8 (40%)*		
30	F	23	2	2.8 (78%)*	1.9 (64%)*	2.3 (74%)*		
58	M	32	4	1.6 (36%)*	0.8 (23%)*	1.1 (32%)*	6	Y
66	F	21	3	3.0 (93%)	2.1 (84%)	2.4 (96%)	17	Y
86	M	26	2	3.8 (92%)	1.9 (61%)*	2.2 (70%)*		
64	F	39	4	2.2 (63%)*	1.3 (63%)*	1.5 (57%)*	78	Y
20	F	26	2	5.9 (126%)	4.3 (108%)	4.8 (121%)		
66	M	28	3	2.8 (64%)*	1.5 (47%)*	2.0 (62%)*		
59	F	39	5	1.9 (59%)*	1.4 (49%)*	1.7 (63%)*	677	Y
35	F	23	5	4.6 (109%)	2.7 (76%)*	3.0 (85%)	790	Y
34	F	33	3	4.3 (120%)	3.3 (110%)	3.7 (124%)		
75	F	24	3	2.1 (82%)	1.3 (71%)*	1.4 (77%)	50	
7	M	20	2	2.0 (97%)	1.5 (88%)	1.8 (105%)	357	
9	F	18	3	2.1 (101%)	1.7 (89%)	1.7 (93%)	334	Y
9	F	18	5	1.6 (81%)	1.1 (63%)*†	1.5 (90%)	1302	Y
11	M	20	5	1.4 (81%)	0.8 (49%)*†	1.4 (93%)	212	Y
14	M	15	5	1.7 (77%)*†	1.0 (49%)*†	1.3 (63%)*†	351	Y
11	M	16	3	2.8 (112%)	2.2 (103%)	2.2 (103%)		Y
60	F	33	3	2.3 (81%)	1.7 (78%)	1.8 (84%)	127	Y
59	F	22	3	2.0 (97%)	1.3 (86%)	1.5 (99%)	<2	

BMI, Body mass index; F, female; M, male.

*Denotes values less than the predicted 95% CI by using National Health and Nutrition Examination Survey NHANES III predicted values for normal based on anthropometric data.^{E1}

†Values less than the lower limit of normal using the Global Lung Initiative calculator for pediatric patients.^{E2}