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1 **Fibroblast-specific integrin alpha V differentially regulates type 17 and type 2 driven**
2 **inflammation and fibrosis**

3 Running Title: Fibroblast-specific integrin-alpha V regulates inflammation and fibrosis

4

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7

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19

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22 analyzed the data; N.C.H provided mice; J.C.S., R.L.G., T.A.W., E.P.K., K.M.V., and K.M.H. wrote the
23 manuscript.

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25 this manuscript was done while at the NIH; however, they have since moved to Pfizer.

26 **Abstract:**

27 Fibroproliferative diseases affect a significant proportion of the world's population. Despite this,
28 core mechanisms driving organ fibrosis of diverse etiologies remain ill defined. Recent studies
29 suggest integrin-alpha V serves as a master driver of fibrosis in multiple organs. Although diverse
30 mechanisms contribute to the progression of fibrosis, TGF- β and IL-13 have emerged as central
31 mediators of fibrosis during type 1/type 17, and type 2 polarized inflammatory responses,
32 respectively. To investigate if integrin-alpha V interactions or signaling is critical to the
33 development of type 2 fibrosis, we analyzed fibroblast-specific integrin-alpha V knockout mice in
34 three type 2-driven inflammatory disease models. While we confirmed a role for integrin-alpha V
35 in type 17-associated fibrosis, integrin-alpha V was not critical to the development of type 2-
36 driven fibrosis. Additionally, our studies support a novel mechanism through which fibroblasts, via
37 integrin-alpha V expression, are capable of regulating immune polarization. We show that when
38 integrin-alpha V is deleted on fibroblasts, initiation of type 17 inflammation is inhibited leading to
39 a deregulation of type 2 inflammation. This mechanism is most evident in a model of severe
40 asthma, which is characterized by a mixed type 2/type 17 inflammatory response. Together, these
41 findings suggest dual targeting of integrin-alpha V and type 2 pathways may be needed to
42 ameliorate fibrosis and prevent rebound of opposing pro-fibrotic and inflammatory mechanisms.

43

44 **Key words:**

45 Fibrosis, Inflammation, Asthma, Liver, Lung, Type 2, Type 17, Th2, Th17

46 **Introduction:**

47 Progressive tissue fibrosis is one of the leading causes of morbidity and mortality worldwide.
48 Despite this, there are few effective approaches to treat fibroproliferative diseases, and currently,
49 the only option for some end stage fibrotic diseases is organ transplantation. Transforming growth
50 factor- β (TGF- β) is a potent family of cytokines with pleiotropic effects, including the ability to
51 influence T cell differentiation, suppresses inflammatory responses by acting on various
52 leukocytes, and to induce fibrosis through the activation of collagen producing myofibroblasts (1).
53 Direct targeting of TGF- β systemically is not generally viewed as therapeutically viable due to off
54 target effects; therefore, the upstream and downstream mediators that control TGF- β activation
55 and function have been studied intensively for their potential in anti-fibrotic therapies (2-4).
56 Integrins are one such upstream target, with accumulating evidence suggesting they may serve as
57 a therapeutic target to ameliorate collagen deposition in fibrotic diseases. During wound repair,
58 integrins expressed by both resident cells and cells migrating towards chemoattractants released
59 at the site of tissue injury can bind to and shear active TGF- β from the latency associated peptide
60 through the contraction of actin-myosin stress fibers (5, 6). Integrins exist as cell surface
61 heterodimers, composed of one alpha and one beta subunit that are able to bind to and interact
62 with the extracellular matrix (7). Of the potential alpha subunits, only alpha V integrin (Itgav) has
63 been shown to be critical for binding and activating TGF- β (5, 8). Variation within the beta subunit
64 of the integrin dimer, by contrast, confers tissue specificity (9). Studies targeting Itgav on
65 myofibroblasts have identified Itgav as a core molecular pathway regulating fibrosis in CCl₄-driven
66 liver fibrosis, bleomycin-induced lung fibrosis, unilateral ureter obstruction-induced kidney
67 fibrosis, and cardiac and skeletal muscle fibrosis, models largely driven by type 17 immune
68 responses (10, 11). These studies identified integrin-mediated TGF- β activation as a promising
69 target for fibroproliferative disease; hence, drugs targeting Itgav are in development.

70

71 We and others have previously shown that type 2 immunity, characterized by the effector
72 cytokines IL-4, IL-5, IL-9, and IL-13, can induce fibrosis through TGF- β -dependent and independent
73 mechanisms (12-14). Nevertheless, the role of integrins in the development of fibrosis during a
74 more polarized type 2 cytokine driven inflammatory response is largely uncharacterized.
75 Therefore, we tested the role of Itgav in several Th2-driven murine models of fibrosis including
76 infection with the helminth *Schistosoma mansoni*, an *S. mansoni* egg-induced pulmonary
77 granuloma model, and a model of severe asthma characterized by mixed type 2/type 17
78 inflammation (15). We found that in the presence of a strong type 2 fibrotic stimulus, deletion of
79 Itgav had no effect on collagen deposition, suggesting the role of Itgav in fibrosis is limited to type
80 17-driven disease entities. Beyond the role of Itgav in fibrosis, we observed marked alterations in
81 type 2 and type 17 inflammation, suggesting a novel mechanism through which stromal cells are
82 capable of shaping the local inflammatory milieu. Specifically, we show that Itgav on fibroblasts is
83 important for the induction of type 17 inflammation. When Itgav is depleted on fibroblasts, type
84 17 inflammation is significantly reduced, leading to a marked increase in type 2 inflammation.
85 These findings have important therapeutic implications as they suggest Itgav targeting alone will
86 not impact fibrosis associated with type 2 inflammation, and may in some conditions exacerbate
87 these pathologies.

88

89 **Results:**

90 **Fibroblast-specific Itgav deletion is not universally protective in models of liver fibrosis**

91 To study the role of Itgav on fibroblasts in a type 2 inflammatory setting we utilized
92 *Pdgfrb^{cre/wt}Itgav^{flox/flox}(Pdgfrb-cre⁺)* and *Itgav^{flox/flox}(Pdgfrb-cre⁻)* mice previously characterized (11).
93 Platelet derived growth factor receptor beta (Pdgfrb) is specifically expressed at high levels by
94 both activated and quiescent fibroblasts, making *Pdgfrb-cre* a useful tool to target deletion of
95 Itgav specifically to fibroblast populations. To investigate the role of Itgav in type 2-driven liver

96 fibrosis *Pdgfrb-cre⁺* and *Pdgfrb-cre⁻* littermates were infected with *S. mansoni* percutaneously via
97 the tail with 35 cercariae (Figure 1A). Livers from these mice were harvested and analyzed at 12
98 weeks post infection following establishment of chronic disease with substantial fibrosis. Fibrosis
99 was unchanged in *Pdgfrb-cre⁺* when compared to *Pdgfrb-cre⁻* as observed by gross picosirius red
100 staining (PSR), quantification of PSR staining, hydroxyproline content, as well as expression of
101 collagen associated genes *Col3a1* and *Col6a1* (Figures 1B-E).

102
103 Previous studies used CCl₄-driven liver fibrosis, a type 17-/TGF- β -driven liver fibrosis model, to
104 demonstrate the critical role of fibroblast-specific *Itgav* in the development of fibrosis (11, 16). To
105 reconfirm the role of *Itgav* in type 17-driven liver disease *Pdgfrb-cre⁺* and *Pdgfrb-cre⁻* littermates
106 were injected intraperitoneally with 1 μ L per gram body weight CCl₄ twice weekly for 6 weeks
107 (Figure 1F). We confirmed that *Pdgfrb-cre⁺* mice showed a dramatic reduction in fibrosis as
108 observed through PSR staining, PSR quantification, hydroxyproline quantification, and *Col1a1* and
109 *Col3a1* expression (Figures 1G-J) when compared to *Pdgfrb-cre⁻* littermates receiving the same
110 treatment. These data suggest that in the liver, type 2-driven fibrosis bypasses the pro-fibrotic
111 mechanisms of *Itgav* and that this mechanism may be specific to type 17-driven disease insults.

112

113 **Fibroblast-specific *Itgav* deletion is not protective in models of type 2-driven lung fibrosis**

114 To determine the broader applicability of these results to other organ systems, we utilized an
115 established secondary pulmonary granuloma model that produces a type 2-driven lung disease
116 similar to that seen in the liver during *S. mansoni* infection. *Pdgfrb-cre⁺* and *Pdgfrb-cre⁻* littermates
117 were sensitized intraperitoneally with 5,000 *S. mansoni* eggs and then challenged 14 days later
118 intravenously with 5,000 live *S. mansoni* eggs by tail injection, which leads to deposition of eggs in
119 the pulmonary capillary bed. Lungs were harvested 7 days post challenge at the peak of the
120 inflammatory response (Figure 2A). *Itgav* deletion on fibroblasts resulted in no differences in

121 granulomatous lung fibrosis, as measured by PSR staining, hydroxyproline content, and *Col3a1*
122 and *Col6a1* mRNA expression in the lung tissue (Figures 2B-D).

123

124 We next confirmed the importance of *Itgav* in type 17/ TGF- β -driven fibrosis in the lung using the
125 bleomycin lung injury model (17). To do so, *Pdgfrb-cre⁺* and *Pdgfrb-cre⁻* littermates were
126 administered 1.5 U/kg of bleomycin intranasally, and their lungs were harvested 28 days post
127 challenge (Figure 2F). Fibrosis was significantly reduced in the *Pdgfrb-cre⁺* animals as observed by
128 PSR staining and hydroxyproline content (Figures 2G, 2H). These results expand our findings in the
129 liver (Figure 1) to the lung, showing that *Itgav* is critical to type 17-driven fibrosis but is bypassed
130 in type 2-driven disease models.

131

132 ***Itgav* deletion on fibroblasts causes alterations in immune polarization in both the lung and liver**

133 Fibroblast-specific *Itgav* has previously been shown to drive fibrosis through TGF- β activation. In
134 addition to its role in fibrosis, TGF- β is a potent immunoregulatory cytokine and an important
135 differentiation factor for Th17 cells. Therefore, immunophenotypic analysis was performed to
136 determine if fibroblasts, via expression of *Itgav*, regulate the local inflammatory milieu. *Pdgfrb-*
137 *cre⁺* and *Pdgfrb-cre⁻* littermates were infected with *S. mansoni* and immune responses were
138 characterized 12 weeks post-infection in the liver (Figure 3A). Liver leukocytes were collected and
139 re-stimulated ex-vivo for analysis by intracellular cytokine staining and flow cytometry. The
140 frequency of IL-17A and IL-13 producing CD4⁺ T cells were not significantly altered in *Pdgfrb-cre⁺*
141 compared *Pdgfrb-cre⁻* mice (Figure 3B). We also assessed cellular determinants associated with
142 type 2 and type 17 inflammation, namely tissue eosinophil and neutrophil accumulation,
143 respectively. We did not detect any significant differences in liver neutrophils between the *Pdgfrb-*
144 *cre⁺* and *Pdgfrb-cre⁻* animals. However, in mice lacking *Itgav* on fibroblasts, eosinophil
145 accumulation was significantly increased (Figure 3B).

146

147 We also performed immune characterization in lungs from animals subjected to the secondary
148 pulmonary granuloma model (Figure 3C). The frequency of IL-13 producing CD4⁺ T cells was not
149 significantly altered in the *Pdgfrb-cre*⁺ compared to *Pdgfrb-cre*⁻ mice. However, the frequency of
150 IL-17A-producing CD4⁺ T cells was significantly reduced in the *Pdgfrb-cre*⁺ compared to *Pdgfrb-cre*⁻
151 animals (Figure 3D). While we did not detect significant differences in lung neutrophils between
152 the *Pdgfrb-cre*⁺ and *Pdgfrb-cre*⁻ animals, overall frequencies were decreased relative to naïve
153 animals due to the overwhelming induction of type 2 inflammation in this response (of CD4⁺
154 population: 23.54% T_H2 vs 0.81% T_H17). However, in mice lacking *Itgav* on fibroblasts, eosinophil
155 accumulation was again significantly increased (Figure 3D). Together, these findings (Figure 3A-D)
156 suggest fibroblast-specific *Itgav* alters local inflammation in type 2-driven liver and lung disease.

157

158 To expand these findings to type 17-driven models, the CCl₄ liver fibrosis model was utilized
159 (Figure 3E). Analysis of the inflammatory readouts in this model demonstrated a reduction in IL-
160 17A-producing CD4⁺ T cells in *Pdgfrb-cre*⁺ animals, and a concomitant increase in IL-13-producing
161 CD4⁺ T cells. We did not observe changes in neutrophil or eosinophil cell frequencies (Figure 3F).

162

163 To investigate if *Itgav* on fibroblasts influences inflammatory character in type 17-driven lung
164 disease, the bleomycin-induced lung fibrosis model was used (Figure 3G). No significant
165 differences in the frequency of IL-17A-producing CD4⁺ T cells from re-stimulated lung leukocytes
166 were observed. However, the frequency of IL-13 producing CD4⁺ T cells increased significantly in
167 *Pdgfrb-cre*⁺ animals. Nevertheless, analysis of infiltrating cell populations revealed a reduction in
168 both Ly6G⁺ neutrophils and Siglec-F⁺ eosinophils in the bronchoalveolar lavage (Figure 4H).

169

170 These data suggest that while presence of Itgav on fibroblasts may not universally impact the
171 development of fibrosis, it can regulate the balance between type 2 and type 17 inflammation,
172 which we and others have demonstrated can play a critical role in the lung (18). Additionally, we
173 determined that the ability of Itgav to alter inflammation is not limited to type 17 models, as
174 similar inflammatory changes are seen in both type 17 and type 2 disease models. Nevertheless,
175 given that the bleomycin and CCl₄ models of fibrosis do not depend on antigen driven T cell
176 activation and associated cytokine responses, as seen in other models used here including the
177 helminth infections, our ability to detect consistent shifts in type-1/type-2 inflammation with
178 these models may be more difficult to discern.

179

180

181 **Itgav deletion on fibroblasts inhibits type 17 inflammation and induces a compensatory type 2**
182 **response in cGMP/HDM asthma model**

183 To this end, we took advantage of a model of severe asthma driven by intranasal instillation of low
184 dose house dust mite extract (HDM) combined with the STING ligand cyclic-di-GMP (cGMP). Unlike
185 the *S. mansoni*, bleomycin, and CCl₄ models, this model has high levels of type 2/type 17 mixed
186 inflammation, making it ideal to investigate the inflammatory changes observed when Itgav is
187 deleted on fibroblasts (15). *Pdgfrb-cre*⁺ and *Pdgfrb-cre*⁻ littermates were sensitized with HDM and
188 cGMP and subsequently challenged with HDM and a lower dose of cGMP (Figure 4A). While we
189 did not detect any differences in fibrosis measured by hydroxyproline between *Pdgfrb-cre*⁺ and
190 *Pdgfrb-cre*⁻ animals (Figure 4B), the type 17 response was significantly decreased in *Pdgfrb-cre*⁺
191 animals as assessed by the frequency of IL-17A-producing CD4⁺ T cells in the lung as well as total
192 tissue IL-17A expression (Figures 4C, 4D). To determine if this corresponded with a functional
193 decrease in the IL-17A pathway, we assessed lung expression of the IL-17A responsive neutrophil
194 chemoattractant genes *Cxcl1* and *Cxcl5*, and observed significant reductions in both (Figure 4E).

195 Additional analysis of infiltrating tissue and BAL cell populations demonstrated significantly fewer
196 tissue and BAL neutrophils in *Pdgfrb-cre⁺* animals in agreement with the decreased expression of
197 the neutrophil chemoattractants (Figure 4F).

198

199 We next asked if the impaired initiation of type 17 inflammation observed in animals with *Itgav*
200 deletion on fibroblasts was accompanied by a dysregulation and increased presence of type 2
201 inflammation as we have previously observed in a chronic HDM model of allergic asthma when
202 mice were treated with a neutralizing mAb to IL-17A (18). Indeed, compared with *Pdgfrb-cre⁻*
203 animals, *Pdgfrb-cre⁺* mice exhibited an increase in type 2 inflammatory markers as measured by
204 significant increases in IL-13⁺ CD4⁺ T cells isolated from lung tissue of the cGMP/HDM mice as well
205 as total tissue *Il13* expression (Figures 4G, 4H). The increased IL-13 response also correlated with
206 increased eosinophil accumulation in both lung tissue and BAL (Figure 4I), increased expression of
207 the eosinophil chemoattractant *Ccl11* and the type 2 immune marker *Chi3l3*. Additionally, IL-13-
208 induced mucus-associated genes, *Muc5ac* and *Gob5* were increased in the lung tissue of *Pdgfrb-
209 cre⁺* animals (19-21) (Figure 4J). Mucus production was also increased, as assessed by AB/PAS
210 tissue staining (Figure 4K). Additionally, through flexiVent analysis, we observed a significant
211 reduction in lung resistance when *Itgav* was deleted on fibroblasts, suggesting improved airway
212 hyperreactivity (AHR) (supplemental figure 1A). These data indicate that fibroblast specific
213 deletion of *Itgav* dysregulates the inflammatory environment, favoring type 2 inflammation,
214 reducing airway resistance but ultimately resulting in worsening of disease-associated eosinophilia
215 and mucus production. The observed reduction in the type 17-signature accompanied by
216 increased type 2-inflammation and mucus production reveals a novel, unexpected, yet pleotropic
217 role for fibroblasts in regulating inflammation, immune balance, and measures of clinically
218 relevant disease severity.

219

220 **Itgav deletion on fibroblasts in conjunction with IL-13 neutralization inhibits Th17 inflammation**
221 **while inhibiting a compensatory Th2 response in cGMP/HDM asthma model**

222 In order to better understand the mechanism underlying fibroblast processing of TGF- β through
223 Itgav and its regulation of the immune response, we asked how *Pdgfrb-cre⁺* animals would behave
224 if the rebound type 2 inflammation was blocked using an IL-13-specific antibody in the same
225 cGMP/HDM model of severe asthma. *Pdgfrb-cre⁺* and *Pdgfrb-cre⁻* littermates were treated with
226 either anti-IL-13 or an IgG isotype control twice weekly (Figure 5A). No changes in fibrosis between
227 the *Pdgfrb-cre⁺* and *Pdgfrb-cre⁻* animals were observed, and anti-IL-13 treatment did not
228 significantly affect fibrosis as shown by hydroxyproline quantification (Figure 5B). We observed
229 the same trend of decreased type 17 inflammation in *Pdgfrb-cre⁺* animals as seen in previous
230 experiments (Figure 4), with anti-IL-13 antibody treatment having minimal effect on the type 17
231 signature as assessed by total tissue *Il17a* expression, frequency of IL-17A-producing CD4⁺ T-cells
232 in the lung, total tissue and BAL neutrophil quantification, and expression of neutrophil
233 chemoattractants *Cxcl1* and *Cxcl5* (Figures 5C-F).

234
235 We next assessed what effect anti-IL-13 treatment would have on the rebound type 2
236 inflammatory response seen in *Pdgfrb-cre⁺* animals in the previous experiments (Figure 4). Again,
237 we observed that the type 2-signature was significantly increased in the absence of Itgav.
238 However, IL-13 antibody treatment ablated this compensatory increase in *Pdgfrb-cre⁺* mice as
239 measured by total tissue *Il13* expression, frequencies of IL-13⁺ CD4⁺ T-cells, total lung tissue and
240 BAL eosinophil frequency, the eosinophil chemoattractant *Ccl11*, and the mucus-associated gene
241 *Muc5ac* (Figures 5G-I). The increased mucus production in *Pdgfrb-cre⁺* animals receiving
242 cGMP/HDM treatment was also reduced in anti-IL-13 treated animals (Figure 5J). Together, these
243 data reveal an important role for fibroblast-specific Itgav expression in the coordination and
244 regulation of type 17 and type 2–driven inflammatory responses.

245

246 **Discussion:**

247 Recent publications have shown Itgav to be part of a fundamental pathway regulating fibrosis in
248 the liver, lung, skin, kidney, and cardiac and skeletal muscle (9). Blockade of either Itgav or one of
249 its five beta subunits, via genetic deletion or chemical blockade, substantially attenuated fibrosis
250 (9, 11). In these studies, Itgav blockade was efficacious both prophylactically and therapeutically.
251 However, these previous studies did not explore the importance of Itgav in settings where type 2
252 cytokines are believed to function as important drivers of disease progression. In this study, we
253 compared mice with or without Itgav on fibroblasts and observed that type 2 fibrosis driven by *S.*
254 *mansoni* infection or egg-induced granuloma formation is independent of fibroblast-specific Itgav
255 expression in the liver and the lung, respectively. In these type 2-driven disease models, integrin
256 activation of TGF- β by fibroblasts played little to no detectable role in fibrosis. Nevertheless, we
257 confirmed that fibrosis driven predominantly by type-17/TGF- β , including bleomycin- and CCl₄-
258 driven fibrosis, were highly Itgav dependent (17).

259

260 Unexpectedly, however, we discovered that Itgav deficiency on fibroblasts resulted in a
261 substantial decrease in type 17 inflammation and corresponding increase in type 2 inflammation,
262 particularly when a mixed type 17/type 2 inflammatory response was observed. The shift from
263 type 17/neutrophilic to type 2/eosinophilic inflammation was most striking in the cGMP/HDM
264 model of severe asthma, which was characterized as mixed type 17/type 2 inflammatory reaction
265 (15). Similarly, a recent publication by Choy et al. showed that Th2 and Th17 inflammatory
266 pathways are reciprocally regulated in both murine and human asthmatic lungs characterized by
267 infiltration of their associated cellular determinants eosinophils and neutrophils, respectively. The
268 results presented here suggest that the balance of these inflammatory pathways can be regulated,
269 in part, by fibroblast-specific Itgav expression. Robust regulation of type-17 inflammatory readouts

270 mediated by Itgav or Itgb8 expression on dendritic cells has been described previously in EAE as
271 well as several asthma models, and was associated with significantly reduced AHR (22-24).
272 However, there were no reported changes in mucus or neutrophil and eosinophil counts despite a
273 near complete inhibition of Th17 development. Surprisingly, despite less dramatic reductions in
274 Th17 differentiation, fibroblast deletion of Itgav resulted in similar reductions in AHR as the Itgb8
275 deletion on dendritic cells that was previously reported. Similarly, global blockade of avb8 with a
276 neutralizing antibody was shown to inhibit IL-17, BAL neutrophils and AHR in a smoke inhalation
277 model; although, it is unclear whether these effects were mediated via dendritic cells, fibroblasts,
278 or both (25). Additional studies by Kitamura et al. demonstrated fibroblast deletion of Itgb8
279 resulted in decreased lung neutrophils and Th17 differentiation in response to adenoviral IL-1b
280 and reduced inflammation and mucus in an ovalbumin challenge lung model that was not
281 associated with decreased IL-17 or changes in neutrophils (26). AHR was not measured in those
282 experiments and it is unclear if the additional modulation of the type-2 response, downstream
283 cellular determinants and mucus we report are specific to the disease model or are mediated thru
284 an alternate beta integrin. Taken together these data suggest some potential similarities in
285 mechanisms regulating type-17 inflammation employed by dendritic cells and fibroblasts via
286 integrins, but they are also suggestive of important differences in cell specific roles that may
287 depend on Itgav pairing partners and disease context.

288

289 This immune regulation by fibroblasts via Itgav appears to be similar as was recently described for
290 dendritic cells (23, 24). Our data demonstrate a role for fibroblasts regulating not only the
291 intensity but also the balance of inflammatory pathways through integrin expression, expanding
292 on a previous study invoking an Itgb8-mediated role for lung fibroblasts in the induction of
293 inflammation in response to IL-1 β (26). The inflammatory changes detected in *Pdgfrb-cre*⁺ mice
294 may be the result of reduced Th17 induction and a compensatory induction and deregulation of

295 the type 2 response. Furthermore, TGF- β is known to support the differentiation of Th0 cells into
296 Th17 cells and regulatory T cells (27-29). Therefore, the reduction of a major source of active TGF-
297 β through Itgav deletion on fibroblasts may impair Th17 differentiation and license these cells
298 towards other lineages. These findings posit a novel role for fibroblasts in the immune polarization
299 decision-making process through Itgav expression.

300

301 These findings also have important therapeutic implications as they suggest targeting Itgav may
302 inadvertently skew inflammatory responses towards type 2 inflammation and alternative pro-
303 fibrotic mechanisms, particularly when stimuli promoting these alternative pathways are also
304 present. Previous studies by our group demonstrated that dual blockade of IL-13 and IL-17A in
305 preclinical asthma models is more efficacious because it limits the rebound inflammation that
306 occurs during mono-therapy. Similarly, in the cGMP/HDM model used in this current study,
307 increased type 2 responses in the absence of fibroblast-specific Itgav led to increased eosinophilia
308 and mucus production, key functional contributors to severe asthmatic disease (30-34). Our
309 flexiVent data demonstrate that targeting Itgav on fibroblasts decreases airway resistance in the
310 cGMP/HDM severe asthma model. This agrees with some reports that neutrophils, not eosinophils,
311 may be the critical drivers of AHR (35-39). Nevertheless, despite the improvements in AHR,
312 concomitant upregulation of type 2 immune responses resulted in significant eosinophilia and
313 mucus production. Mucus hypersecretion is a hallmark of chronic airway diseases, including
314 asthma, chronic obstructive pulmonary disease, and cystic fibrosis. Studies examining the effect of
315 mucus on lung pathology show that mucus overproduction is directly correlated to progressive
316 declines in lung function, worsened quality of life, hospitalizations, and mortality (40-56).
317 Therefore, we believe that while AHR may be decreased, the induction of mucus overproduction is
318 of clinical importance and should be considered during treatment and clinical trial development.
319 Overall, these results indicate that while fibroblast-specific expression of Itgav is a critical driver of

320 Th17 inflammation in the asthmatic lung, therapeutic targeting of Itgav may require a coordinated
321 treatment strategy to prevent compensatory rebound type 2 inflammation. Increasing evidence
322 suggests that the emergent type 17 inflammation in treatment-resistant severe asthma may be
323 associated with potent corticosteroid control of the type 2 response (18, 57, 58). The effectiveness
324 of Itgav deletion on fibroblasts to control Th17 induction indicates it may serve as an ideal paired
325 therapy with standard therapy or novel blockers of type 2 inflammation. Indeed, in our studies,
326 administration of anti-IL-13 to animals lacking Itgav on fibroblasts reduced eosinophilia and mucus
327 production to levels of saline controls. These data suggest that dual blockade of Itgav and IL-13, or
328 other mediators of type 2 immunity may represent a more efficacious therapeutic strategy for
329 treatment-resistant asthmatics.

330

331 We conclude that in fibrotic diseases of diverse etiologies, the role of fibroblast-specific Itgav in
332 fibrosis is highly dependent on the inflammatory stimuli driving the response. In addition, these
333 data reveal a new role for Itgav expression on stromal cells, as they show it plays a critical role in
334 shaping the character of local inflammatory responses. Thus, while targeting Itgav in fibrotic
335 disease may be efficacious in some settings, it will be important to understand the inflammatory
336 mechanisms driving the fibrogenic response in order to better predict clinical outcome. Our
337 findings suggest Itgav inhibition may be utilized to attenuate type 17 inflammation in diseases
338 such as severe asthma. While type-17 inflammatory pathways may be downregulated by Itgav
339 blockade, the potential for an undesirable augmentation of type-2 inflammation and resulting
340 pathology should be monitored carefully. Indeed, in settings where there is high risk of
341 compensatory type 2 inflammation, dual blockade strategies may be required to maximize
342 therapeutic benefit.

343

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350 manuscript was done while at the NIH; however, they have since moved to Pfizer, Cambridge, MA.

351

352 **Materials and Methods:**

353 **Mice**

354 *Pdgfrb^{cre/wt}Itgav^{flox/flox}* were obtained from Neil Henderson (11); animals used in studies were age
355 and gender matched, littermates and between 6-8 weeks of age. Mice were terminally
356 anesthetized with sodium pentobarbital. All animals were housed under specific pathogen-free
357 conditions at the National Institutes of Health in an American Association for the Accreditation of
358 Laboratory Animal Care-approved facility.

359

360 ***Schistosoma mansoni* Infection**

361 Mice were infected percutaneously by suspending tails in pond water containing 35 *S. mansoni*
362 cercariae for 45 minutes. Cercariae were obtained by shedding infected *Biomphalaria glabrata*
363 snails (Biomedical Research Institute).

364

365 **Pulmonary Granuloma Model**

366 *S. mansoni* eggs were extracted from the livers of infected mice (Biomedical Research Institute,
367 Rockville, MD, USA). For the induction of secondary lung granulomas, mice were sensitized
368 intraperitoneally (i.p.) with 5,000 *S. mansoni* eggs and then challenged 14 days later with 5,000
369 live *S. mansoni* eggs i.v.

370

371 **CCl₄ and Bleomycin Fibrosis Models**

372 For chronic CCl₄-induced liver fibrosis, mice were injected i.p. with 1 μl CCl₄ (Sigma)/gram body
373 weight or olive oil twice a week for 6 weeks. To induce pulmonary fibrosis, mice were
374 anesthetized, and saline or with 1.5 U bleomycin (Sigma)/kg body weight was instilled
375 intratracheally.

376

377 **cGMP/HDM Model of Severe Asthma**

378 Mice were sensitized to 25 μg HDM (greer labs) extract and 5 μg cyclic-di-GMP (cayman chemical
379 company) delivered intranasally on days 1, 3, and 5. Mice were rested 5 days, then intranasally
380 administered with 3 challenge sets consisting of 3 consecutive daily challenges with HDM and
381 cyclic-di-GMP with 4 days of rest between each set. Each challenge set included 0.5 μg cyclic-di-
382 GMP with 25 μg HDM on day 1, then 25 μg HDM on the following 2 days.

383

384 **Histopathology**

385 Murine liver and lung lobes were harvested and fixed with Bouin's-Hollande solution. Fixed tissue
386 was embedded in paraffin for sectioning, and stained (Histopath of America, Clinton, MD) with
387 Wright's Giemsa, Picrosirius red (PSR), or Alcian Blue Periodic Acid Schiff (AB/PAS).

388

389 **Fibrosis Quantification**

390 Cross sections of three liver lobes from each mouse were stained with PSR and imaged under
391 polarized light. Granuloma volume was obtained by measuring the length (a) and width (b) of
392 granulomas with a single visible egg. Values obtained were entered into the formula of a spheroid

393 $(\frac{4}{3}\pi ab^2)$.

394

395 **IL-13 Neutralization**

396 For antibody therapy, 250 µg of anti-IL-13 (Genentech clone 262A-5-1), or control Ab (BioXCell
397 clone MOPC-21, catalog #BE0083) was injected intraperitoneally twice weekly.

398

399 **Hydroxyproline Assay**

400 Mouse liver tissue (200 mg) or the left lower lung lobe incubated overnight at 110 °C in 6M HCl.
401 Hydroxyproline content was measured using a colorimetric chloramine T assay (59).

402

403 **Cell Isolation and Flow Cytometry**

404 Approximately 400 mg of liver or lung tissue was diced and incubated in 100 U/ml of collagenase
405 (Sigma) at 37°C for an hour with rocking. Tissue was then passed through a 70-µm nylon filter to
406 obtain a single cell suspension. Leukocytes were isolated on a 40% Percoll (Sigma) gradient, and
407 treated with ACK lysis buffer to remove erythrocytes. Isolated cells were either immediately
408 stained for cellular analysis or stimulated with phorbol 12-myristate 13-acetate (10ng/ml) and
409 ionomycin (1 µg/ml) in the presence of Brefeldin A (10 µg/ml) for three hours and fixed. Cells were
410 permeabilized (Cytotfix/Cytoperm buffer; BD Biosciences; San Diego, CA) and stained for 30
411 minutes with fluorescently labelled antibodies purchased from eBioscience (Waltham, MA)
412 include the following: CD45(30-F11; 1:250) and IL-13(eBio13A; 1:150). Antibodies purchased from
413 Biolegend (San Diego, CA) include the following: CD16/CD32 (93; 1:500), CD11b (M1/70; 1:250),
414 and IL-17A(TC11-18H10.1; 1:150). Antibodies purchased from BD Pharmingen (Billerica, MA)
415 include the following: CD4 (RM4-5; 1:150), Ly6C (AL-21; 1:250), ly6G(1A8; 1:350), and Siglec-F
416 (E50-2440; 1:275). Cells were collected on an LSR II flow cytometer equipped with FACSDIVA (BD
417 Biosciences) software and data were analyzed with FlowJo software (Tree Star, Ashland, OR).

418

419 **Murine Gene Expression Analyses**

420 Liver and lung tissue was homogenized in TRIzol Reagent (Life Technologies; Grand Island, NY)
 421 with a Precellys 24 (Bertin Technologies; Montigny-le-Bretonneux, France). Total RNA was
 422 extracted with chloroform using a MagMax-96 Total RNA Isolation Kit (Qiagen), and reverse
 423 transcribed to cDNA using SuperScript II Reverse Transcriptase (Life Technologies). Gene
 424 expression was quantified using Power SYBR Green PCR Master Mix (Applied Biosystems) by RT-
 425 PCR on an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Gene expression is
 426 described relative to RPLP2 mRNA levels in naïve liver and lung tissue.

Gene	Forward Sequence 5' - 3'	Reverse sequence 5'-3'
<i>Ccl11</i>	GAATCACCAACAACAGATGCAC	ATCCTGGACCCACTTCTTCTT
<i>Chil3</i>	CATGAGCAAGACTTGCGTGAC	GGTCCAAACTTCCATCCTCCA
<i>Col3a1</i>	AACCTGGTTTCTTCTCACCTTC	ACTCATAGGACTGACCAAGGTGG
<i>Col6a1</i>	CGCCCTTCCCACTGACAA	GCGTTCCTTTAAGACAGTTGAG
<i>Cxcl1</i>	CTGGGATTCACCTCAAGAAC	GAAGCCAGCGTTCACCAGAC
<i>Cxcl5</i>	TGCGTTGTGTTTGCTTAACCG	AGCTATGACTTCCACCGTAGG
<i>Gob5</i>	AGGAAAACCCCAAGCAGTG	GCACCGACGAACCTTGATTTT
<i>Il13</i>	CCTCTGACCCTTAAGGAGCTTAT	CGTTGCACAGGGGAGTCTT
<i>Il17a</i>	TTTAACTCCCTTGCGCAAAA	CTTCCCTCCGCATTGACAC
<i>Muc5ac</i>	CAGGACTCTCTGAAATCGTACCA	AAGGCTCGTACCACAGGGA

427

428 **Statistical Analysis**

429 Experimental groups were randomized and researchers were blinded to the groups. Prism 7 was
 430 used to compute statistical analyses. Data was tested for normal distribution and two-tailed
 431 Welch's t test or one-way ANOVA were used to determine statistical significance. A *p* value <0.05
 432 was deemed statistically significant.

433

434 **Ethics**

435 The National Institute of Allergy and Infectious Diseases Division of Intramural Research Animal
 436 Care and Use Program, as part of the National Institutes of Health Intramural Research Program,
 437 approved all of the experimental procedures (protocol "LPD 16E"). The program complies with all
 438 applicable provisions of the Animal Welfare Act

439 (www.aphis.usda.gov/animal_welfare/downloads/awa/awa.pdf) and other federal statutes and
440 regulations relating to animals.

441 **Figure 1. Fibroblast-specific Itgav deletion is not universally protective in models of liver fibrosis**

442 (A) *Pdgfrb*^{cre/wt}*Itgav*^{fllox/fllox}(*Pdgfrb-cre*⁺) and *Itgav*^{fllox/fllox}(*Pdgfrb-cre*⁻) mice were infected with

443 *Schistosoma Mansoni* percutaneously via the tail with 25–35 cercariae. Livers were harvested 12

444 weeks post infection. (B) 5- μ m sections of paraffin-embedded liver tissue were stained with PSR.

445 (C) Quantification of positive PSR staining, expressed as percentage of pixels positive for stain.

446 From left to right: n = 4, 4, 9, 6. (D) Liver collagen deposition, expressed as micromoles of

447 hydroxyproline per gram of liver. From left to right: n = 4, 4, 9, 6. (E) RNA was extracted from liver

448 tissue, with *Col3a1*, and *Col6a1* quantified by quantitative RT-PCR. From left to right: n = 4, 4, 8, 6.

449 (F) *Pdgfrb-cre*⁺ and *Pdgfrb-cre*⁻ mice were injected i.p. with 1 μ l per gram body weight CCl₄ twice

450 weekly for 6 weeks. (G) 5- μ m sections of paraffin-embedded liver tissue were stained with PSR.

451 (H) Quantification of positive PSR staining, expressed as percentage of pixels positive for stain.

452 From left to right: n = 5, 9, 10. (I) Liver collagen deposition, expressed as micromoles of

453 hydroxyproline per liver. From left to right: n = 4, 5, 14, 15. (J) RNA was extracted from lung tissue,

454 with *Col1a1* and *Col3a1*mRNA quantified by quantitative RT-PCR. From left to right: n = 3, 3, 9, 10.

455 Data representative of two replicate experiments; all scale bars represent 100 μ m; *p < 0.05, **p

456 < 0.01, ***p < 0.001.

457 **Figure 2. Fibroblast-specific Itgav deletion is not protective in models of type 2-driven lung**
458 **fibrosis**

459 (A) *Pdgfrb-cre⁺* and *Pdgfrb-cre⁻* mice were sensitized intraperitoneally with 5,000 *S. mansoni* eggs
460 and then challenged 14 days later intravenously with 5,000 live *S. mansoni* eggs. Lungs were
461 harvested 7 days post challenge. (B) 5- μ m sections of paraffin-embedded lung tissue were stained
462 with PSR. (C) Pulmonary collagen deposition, expressed as micromoles of hydroxyproline per lung.
463 From left to right: n = 4, 4, 9, 15. (D) Quantification of granuloma volume. From left to right: n = 4,
464 4, 4, 4. (E) RNA was extracted from lung tissue, with *Col3a1*, and *Col6a1* quantified by quantitative
465 RT-PCR. From left to right: n = 3, 3, 4, 6. (F) *Pdgfrb^{cre/wt}Itgav^{flox/flox}(Pdgfrb-cre⁺)* and
466 *Itgav^{flox/flox}(Pdgfrb-cre⁻)* mice were administered 1.5U bleomycin i.n. and lungs were harvested 28
467 days later. (G) 5- μ m sections of paraffin-embedded lung tissue were stained with picrosirius red.
468 (H) Pulmonary collagen deposition, expressed as micromoles of hydroxyproline per lung. From left
469 to right: n = 6, 6, 12, 9.
470 Data representative of two replicate experiments; all scale bars represent 100 μ m; *p < 0.05, **p
471 < 0.01, ***p < 0.001.

472 **Figure 3. Itgav deletion on fibroblasts causes alterations in immune polarization in both the lung**
473 **and liver**

474 (A) *Pdgfrb^{cre/wt}Itgav^{flox/flox}(Pdgfrb-cre⁺)* and *Itgav^{flox/flox}(Pdgfrb-cre⁻)* mice were infected with
475 *Schistosoma Mansoni* percutaneously via the tail with 25–35 cercariae. Livers were harvested 12
476 weeks post infection (B) Liver lymphocytes were collected from *S. Mansoni* infected animals and
477 re-stimulated ex-vivo for analysis by intracellular staining for IL-13, IL-17A, Ly6G, and Siglec-F and
478 analyzed via flow cytometry. From left to right: n = 4, 4, 8, 6. (C) *Pdgfrb-cre⁺* and *Pdgfrb-cre⁻* mice
479 were sensitized intraperitoneally with 5,000 *S. mansoni* eggs and then challenged 14 days later
480 intravenously with 5,000 live *S. mansoni* eggs. Lungs were harvested 7 days post challenge. (D)
481 Lung lymphocytes were collected from mice, subjected to secondary lung granuloma, model and
482 re-stimulated ex-vivo for analysis by intracellular staining for IL-13, IL-17A, Ly6G, and Siglec-F and
483 analyzed via flow cytometry. From left to right: n = 5, 4, 9, 14. (E) *Pdgfrb-cre⁺* and *Pdgfrb-cre⁻* mice
484 were injected i.p. with 1 μ l per gram body weight CCl₄ twice weekly for 6 weeks. (F) Liver
485 lymphocytes were collected and re-stimulated ex-vivo for analysis by intracellular staining for IL-
486 17A, IL-13, Ly6G, and Siglec-F and analyzed via flow cytometry. From left to right: n = 4, 5, 4. (G)
487 *Pdgfrb^{cre/wt}Itgav^{flox/flox}(Pdgfrb-cre⁺)* and *Itgav^{flox/flox}(Pdgfrb-cre⁻)* mice were administered 1.5U
488 bleomycin i.n. and lungs were harvested 28 days later. (H) Lung lymphocytes were collected and
489 re-stimulated ex-vivo for analysis by intracellular staining for IL-17A, IL-13, Ly6G, and Siglec-F and
490 analyzed via flow cytometry. From left to right: n = 3, 5, 14, 10.

491 Data representative of two replicate experiments; *p < 0.05, **p < 0.01, ***p < 0.001

492 **Figure 4. Itgav deletion on fibroblasts inhibits Th17 inflammation and induces a compensatory**
493 **Th2 response in cGMP/HDM asthma model**

494 (A) *Pdgfrb*^{cre/wt}*Itgav*^{flox/flox}(*Pdgfrb-cre*⁺) and *Itgav*^{flox/flox}(*Pdgfrb-cre*⁻) mice were sensitized with
495 house dust mite allergen and ci-di-GMP and subsequently challenged with HDM and a lower dose
496 of ci-di-GMP. (B) Pulmonary collagen deposition, expressed as micromoles of hydroxyproline per
497 lung. From left to right: n = 6, 7, 12, 13. (C) Lung lymphocytes were collected and re-stimulated ex-
498 vivo for analysis by intracellular staining for IL-17A. From left to right: n = 5, 6, 12, 12. (D-E) RNA
499 was extracted from lung tissue, with *Il17a*, *Cxcl1* and *Cxcl5* mRNA quantified by quantitative RT-
500 PCR. From left to right: n = 5, 6, 11, 12. (F-G) Lung lymphocytes were collected and re-stimulated
501 ex-vivo for analysis by intracellular staining for Ly6G, and IL-13 and analyzed via flow cytometry.
502 From left to right: n = 6, 7, 10, 12. (H) RNA was extracted from lung tissue, with *Il13* mRNA
503 quantified by quantitative RT-PCR. From left to right: n = 4, 4, 5, 5. (I) Lung lymphocytes were
504 collected and re-stimulated ex-vivo for analysis by intracellular staining for Siglec-F and analyzed
505 via flow cytometry. From left to right: n = 6, 7, 10, 12. (J) RNA was extracted from lung tissue, with
506 *Ccl11*, *Chil3*, *Muc5ac* and *Gob5* mRNA quantified by quantitative RT-PCR. From left to right: n = 5,
507 6, 11, 12. (K) 5- μ m sections of paraffin-embedded lung tissue were stained with AB/ PAS. Data
508 representative of two replicate experiments; all scale bars represent 100 μ m; *p < 0.05, **p <
509 0.01, ***p < 0.001.

510 **Figure 5 Itgav deletion on fibroblasts in conjunction with IL-13-neutralization inhibits Th17**
511 **inflammation while inhibiting a compensatory Th2 response in cGMP/HDM asthma model**
512 (A *Pdgfrb*^{cre/wt}*Itgav*^{flox/flox}(*Pdgfrb-cre*⁺) and *Itgav*^{flox/flox}(*Pdgfrb-cre*⁻) mice were sensitized with house
513 dust mite allergen and ci-di-GMP and subsequently challenged with HDM and a lower dose of ci-
514 di-GMP. 250ug of anti-IL-13 was also administered twice weekly. (B) Pulmonary collagen
515 deposition, expressed as micromoles of hydroxyproline per lung. From left to right: n = 6, 6, 11,
516 11, 14, 13. (C) RNA was extracted from lung tissue, with *Il17a* mRNA quantified by quantitative RT-
517 PCR. From left to right: n = 6, 4, 8, 8, 14, 12. (D-E) Lung lymphocytes were collected and re-
518 stimulated ex-vivo for analysis by intracellular staining for IL-17A and Ly6G. From left to right: n =
519 5, 3, 10, 9, 14, 12. (F-G) RNA was extracted from lung tissue, with *Cxcl1*, *Cxcl5*, and *Il13* mRNA
520 quantified by quantitative RT-PCR. From left to right: n = 6, 4, 8, 8, 14, 12. (H) Lung lymphocytes
521 were collected and re-stimulated ex-vivo for analysis by intracellular staining for IL-13 and Siglec-F
522 and analyzed via flow cytometry. From left to right: n = 6, 4, 8, 8, 14, 12. (I) RNA was extracted
523 from lung tissue, with *Ccl11* and *Muc5ac* mRNA quantified by quantitative RT-PCR. From left to
524 right: n = 6, 4, 8, 8, 14, 12. (J) 5- μ m sections of paraffin-embedded lung tissue were stained with
525 AB/PAS. Data representative of two replicate experiments; all scale bars represent 100 μ m; *p <
526 0.05, **p < 0.01, ***p < 0.001.

527 **References:**

- 528 1. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The
529 myofibroblast: one function, multiple origins. *Am J Pathol.* 2007;170(6):1807-16. Epub
530 2007/05/26. doi: 10.2353/ajpath.2007.070112. PubMed PMID: 17525249; PubMed Central
531 PMCID: PMCPMC1899462.
- 532 2. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-beta: the master regulator of fibrosis. *Nat Rev*
533 *Nephrol.* 2016;12(6):325-38. Epub 2016/04/26. doi: 10.1038/nrneph.2016.48. PubMed PMID:
534 27108839.
- 535 3. Rice LM, Padilla CM, McLaughlin SR, Mathes A, Ziemek J, Goummih S, et al. Fresolimumab
536 treatment decreases biomarkers and improves clinical symptoms in systemic sclerosis patients. *J*
537 *Clin Invest.* 2015;125(7):2795-807. Epub 2015/06/23. doi: 10.1172/JCI77958. PubMed PMID:
538 26098215; PubMed Central PMCID: PMCPMC4563675.
- 539 4. Trachtman H, Fervenza FC, Gipson DS, Heering P, Jayne DR, Peters H, et al. A phase 1,
540 single-dose study of fresolimumab, an anti-TGF-beta antibody, in treatment-resistant primary
541 focal segmental glomerulosclerosis. *Kidney Int.* 2011;79(11):1236-43. Epub 2011/03/04. doi:
542 10.1038/ki.2011.33. PubMed PMID: 21368745; PubMed Central PMCID: PMCPMC3257033.
- 543 5. Annes JP, Rifkin DB, Munger JS. The integrin alphaVbeta6 binds and activates latent
544 TGFbeta3. *FEBS Lett.* 2002;511(1-3):65-8. Epub 2002/02/01. PubMed PMID: 11821050.
- 545 6. Wipff PJ, Rifkin DB, Meister JJ, Hinz B. Myofibroblast contraction activates latent TGF-beta1
546 from the extracellular matrix. *J Cell Biol.* 2007;179(6):1311-23. doi: 10.1083/jcb.200704042.
547 PubMed PMID: 18086923; PubMed Central PMCID: PMCPMC2140013.
- 548 7. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell.* 2002;110(6):673-87.
549 Epub 2002/09/26. PubMed PMID: 12297042.
- 550 8. Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, et al. The integrin alpha v
551 beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation
552 and fibrosis. *Cell.* 1999;96(3):319-28. PubMed PMID: 10025398.
- 553 9. Conroy KP, Kitto LJ, Henderson NC. alphav integrins: key regulators of tissue fibrosis. *Cell*
554 *Tissue Res.* 2016;365(3):511-9. doi: 10.1007/s00441-016-2407-9. PubMed PMID: 27139180;
555 PubMed Central PMCID: PMCPMC5010580.
- 556 10. Murray IR, Gonzalez ZN, Baily J, Dobie R, Wallace RJ, Mackinnon AC, et al. alphav integrins
557 on mesenchymal cells regulate skeletal and cardiac muscle fibrosis. *Nat Commun.* 2017;8(1):1118.
558 Epub 2017/10/25. doi: 10.1038/s41467-017-01097-z. PubMed PMID: 29061963; PubMed Central
559 PMCID: PMCPMC5653645.
- 560 11. Henderson NC, Arnold TD, Katamura Y, Giacomini MM, Rodriguez JD, McCarty JH, et al.
561 Targeting of alphav integrin identifies a core molecular pathway that regulates fibrosis in several
562 organs. *Nat Med.* 2013;19(12):1617-24. doi: 10.1038/nm.3282. PubMed PMID: 24216753;
563 PubMed Central PMCID: PMCPMC3855865.
- 564 12. Gieseck RL, 3rd, Ramalingam TR, Hart KM, Vannella KM, Cantu DA, Lu WY, et al.
565 Interleukin-13 Activates Distinct Cellular Pathways Leading to Ductular Reaction, Steatosis, and
566 Fibrosis. *Immunity.* 2016;45(1):145-58. Epub 2016/07/17. doi: 10.1016/j.immuni.2016.06.009.
567 PubMed PMID: 27421703; PubMed Central PMCID: PMCPMC4956513.
- 568 13. Gieseck RL, 3rd, Wilson MS, Wynn TA. Type 2 immunity in tissue repair and fibrosis. *Nat*
569 *Rev Immunol.* 2018;18(1):62-76. Epub 2017/08/31. doi: 10.1038/nri.2017.90. PubMed PMID:
570 28853443.
- 571 14. Ramalingam TR, Gieseck RL, Acciani TH, K MH, Cheever AW, Mentink-Kane MM, et al.
572 Enhanced protection from fibrosis and inflammation in the combined absence of IL-13 and IFN-
573 gamma. *J Pathol.* 2016;239(3):344-54. Epub 2016/04/30. doi: 10.1002/path.4733. PubMed PMID:
574 27125685; PubMed Central PMCID: PMCPMC4915976.

- 575 15. Gauthier M, Chakraborty K, Oriss TB, Raundhal M, Das S, Chen J, et al. Severe asthma in
576 humans and mouse model suggests a CXCL10 signature underlies corticosteroid-resistant Th1 bias.
577 JCI Insight. 2017;2(13). Epub 2017/07/07. doi: 10.1172/jci.insight.94580. PubMed PMID:
578 28679952; PubMed Central PMCID: PMC5499373.
- 579 16. Fabre T, Molina MF, Soucy G, Goulet JP, Willems B, Villeneuve JP, et al. Type 3 cytokines IL-
580 17A and IL-22 drive TGF-beta-dependent liver fibrosis. *Sci Immunol*. 2018;3(28). Epub 2018/10/28.
581 doi: 10.1126/sciimmunol.aar7754. PubMed PMID: 30366940.
- 582 17. Wilson MS, Madala SK, Ramalingam TR, Gochuico BR, Rosas IO, Cheever AW, et al.
583 Bleomycin and IL-1beta-mediated pulmonary fibrosis is IL-17A dependent. *J Exp Med*.
584 2010;207(3):535-52. doi: 10.1084/jem.20092121. PubMed PMID: 20176803; PubMed Central
585 PMCID: PMC2839145.
- 586 18. Choy DF, Hart KM, Borthwick LA, Shikotra A, Nagarkar DR, Siddiqui S, et al. TH2 and TH17
587 inflammatory pathways are reciprocally regulated in asthma. *Sci Transl Med*.
588 2015;7(301):301ra129. doi: 10.1126/scitranslmed.aab3142. PubMed PMID: 26290411.
- 589 19. Hauber HP, Gholami D, Koppermann G, Heuer HE, Meyer A, Pforte A. Increased expression
590 of Interleukin-13 but not Interleukin-4 in cystic fibrosis patients. *J Cyst Fibros*. 2003;2(4):189-94.
591 Epub 2004/10/07. doi: 10.1016/S1569-1993(03)00091-2. PubMed PMID: 15463872.
- 592 20. Miotto D, Ruggieri MP, Boschetto P, Cavallesco G, Papi A, Bononi I, et al. Interleukin-13 and
593 -4 expression in the central airways of smokers with chronic bronchitis. *Eur Respir J*.
594 2003;22(4):602-8. Epub 2003/10/30. PubMed PMID: 14582911.
- 595 21. Whittaker L, Niu N, Temann UA, Stoddard A, Flavell RA, Ray A, et al. Interleukin-13
596 mediates a fundamental pathway for airway epithelial mucus induced by CD4 T cells and
597 interleukin-9. *Am J Respir Cell Mol Biol*. 2002;27(5):593-602. Epub 2002/10/25. doi:
598 10.1165/rcmb.4838. PubMed PMID: 12397019.
- 599 22. Acharya M, Mukhopadhyay S, Paidassi H, Jamil T, Chow C, Kissler S, et al. alphav Integrin
600 expression by DCs is required for Th17 cell differentiation and development of experimental
601 autoimmune encephalomyelitis in mice. *J Clin Invest*. 2010;120(12):4445-52. Epub 2010/11/26.
602 doi: 10.1172/JCI43796. PubMed PMID: 21099114; PubMed Central PMCID: PMC2993596.
- 603 23. Melton AC, Bailey-Bucktrout SL, Travis MA, Fife BT, Bluestone JA, Sheppard D. Expression
604 of alphavbeta8 integrin on dendritic cells regulates Th17 cell development and experimental
605 autoimmune encephalomyelitis in mice. *J Clin Invest*. 2010;120(12):4436-44. Epub 2010/11/26.
606 doi: 10.1172/JCI43786. PubMed PMID: 21099117; PubMed Central PMCID: PMC2993595.
- 607 24. Kudo M, Melton AC, Chen C, Engler MB, Huang KE, Ren X, et al. IL-17A produced by
608 alphabeta T cells drives airway hyper-responsiveness in mice and enhances mouse and human
609 airway smooth muscle contraction. *Nat Med*. 2012;18(4):547-54. Epub 2012/03/06. doi:
610 10.1038/nm.2684. PubMed PMID: 22388091; PubMed Central PMCID: PMC3321096.
- 611 25. Minagawa S, Lou J, Seed RI, Cormier A, Wu S, Cheng Y, et al. Selective targeting of TGF-beta
612 activation to treat fibroinflammatory airway disease. *Sci Transl Med*. 2014;6(241):241ra79. Epub
613 2014/06/20. doi: 10.1126/scitranslmed.3008074. PubMed PMID: 24944194; PubMed Central
614 PMCID: PMC4341974.
- 615 26. Kitamura H, Cambier S, Somanath S, Barker T, Minagawa S, Markovics J, et al. Mouse and
616 human lung fibroblasts regulate dendritic cell trafficking, airway inflammation, and fibrosis
617 through integrin alphavbeta8-mediated activation of TGF-beta. *J Clin Invest*. 2011;121(7):2863-75.
618 doi: 10.1172/JCI45589. PubMed PMID: 21646718; PubMed Central PMCID: PMC3223836.
- 619 27. Ghoreschi K, Laurence A, Yang XP, Tato CM, McGeachy MJ, Konkel JE, et al. Generation of
620 pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature*. 2010;467(7318):967-71.
621 Epub 2010/10/22. doi: 10.1038/nature09447. PubMed PMID: 20962846; PubMed Central PMCID:
622 PMC3108066.

- 623 28. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, et al.
624 TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-
625 mediated pathology. *Nat Immunol.* 2007;8(12):1390-7. Epub 2007/11/13. doi: 10.1038/ni1539.
626 PubMed PMID: 17994024.
- 627 29. Romagnani S, Maggi E, Liotta F, Cosmi L, Annunziato F. Properties and origin of human
628 Th17 cells. *Mol Immunol.* 2009;47(1):3-7. Epub 2009/02/06. doi: 10.1016/j.molimm.2008.12.019.
629 PubMed PMID: 19193443.
- 630 30. Cohn L. Mucus in chronic airway diseases: sorting out the sticky details. *J Clin Invest.*
631 2006;116(2):306-8. Epub 2006/02/03. doi: 10.1172/JCI27690. PubMed PMID: 16453018; PubMed
632 Central PMCID: PMCPMC1359062.
- 633 31. Denlinger LC, Phillips BR, Ramratnam S, Ross K, Bhakta NR, Cardet JC, et al. Inflammatory
634 and Comorbid Features of Patients with Severe Asthma and Frequent Exacerbations. *Am J Respir
635 Crit Care Med.* 2017;195(3):302-13. Epub 2016/08/25. doi: 10.1164/rccm.201602-0419OC.
636 PubMed PMID: 27556234; PubMed Central PMCID: PMCPMC5328178.
- 637 32. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma
638 exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet.*
639 2002;360(9347):1715-21. Epub 2002/12/14. doi: 10.1016/S0140-6736(02)11679-5. PubMed PMID:
640 12480423.
- 641 33. Nakagome K, Nagata M. Involvement and Possible Role of Eosinophils in Asthma
642 Exacerbation. *Front Immunol.* 2018;9:2220. Epub 2018/10/17. doi: 10.3389/fimmu.2018.02220.
643 PubMed PMID: 30323811; PubMed Central PMCID: PMCPMC6172316.
- 644 34. Price DB, Rigazio A, Campbell JD, Bleecker ER, Corrigan CJ, Thomas M, et al. Blood
645 eosinophil count and prospective annual asthma disease burden: a UK cohort study. *Lancet Respir
646 Med.* 2015;3(11):849-58. Epub 2015/10/24. doi: 10.1016/S2213-2600(15)00367-7. PubMed PMID:
647 26493938.
- 648 35. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an
649 interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the
650 late asthmatic response. *Lancet.* 2000;356(9248):2144-8. Epub 2001/02/24. PubMed PMID:
651 11191542.
- 652 36. Fahy JV, Kim KW, Liu J, Boushey HA. Prominent neutrophilic inflammation in sputum from
653 subjects with asthma exacerbation. *J Allergy Clin Immunol.* 1995;95(4):843-52. Epub 1995/04/01.
654 PubMed PMID: 7722165.
- 655 37. Kikuchi S, Nagata M, Kikuchi I, Hagiwara K, Kanazawa M. Association between neutrophilic
656 and eosinophilic inflammation in patients with severe persistent asthma. *Int Arch Allergy
657 Immunol.* 2005;137 Suppl 1:7-11. Epub 2005/06/11. doi: 10.1159/000085425. PubMed PMID:
658 15947478.
- 659 38. Little SA, MacLeod KJ, Chalmers GW, Love JG, McSharry C, Thomson NC. Association of
660 forced expiratory volume with disease duration and sputum neutrophils in chronic asthma. *Am J
661 Med.* 2002;112(6):446-52. Epub 2002/04/18. PubMed PMID: 11959054.
- 662 39. Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in
663 severe persistent asthma. *Am J Respir Crit Care Med.* 1999;160(5 Pt 1):1532-9. Epub 1999/11/11.
664 doi: 10.1164/ajrccm.160.5.9806170. PubMed PMID: 10556116.
- 665 40. Annesi I, Kauffmann F. Is respiratory mucus hypersecretion really an innocent disorder? A
666 22-year mortality survey of 1,061 working men. *Am Rev Respir Dis.* 1986;134(4):688-93. Epub
667 1986/10/01. doi: 10.1164/arrd.1986.134.4.688. PubMed PMID: 3767125.
- 668 41. de Oca MM, Halbert RJ, Lopez MV, Perez-Padilla R, Talamo C, Moreno D, et al. The chronic
669 bronchitis phenotype in subjects with and without COPD: the PLATINO study. *Eur Respir J.*
670 2012;40(1):28-36. Epub 2012/01/28. doi: 10.1183/09031936.00141611. PubMed PMID: 22282547.

671 42. Guerra S, Sherrill DL, Venker C, Ceccato CM, Halonen M, Martinez FD. Chronic bronchitis
672 before age 50 years predicts incident airflow limitation and mortality risk. *Thorax*.
673 2009;64(10):894-900. Epub 2009/07/08. doi: 10.1136/thx.2008.110619. PubMed PMID:
674 19581277; PubMed Central PMCID: PMC4706745.

675 43. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic
676 obstructive lung disease. *N Engl J Med*. 1968;278(25):1355-60. Epub 1968/06/20. doi:
677 10.1056/NEJM196806202782501. PubMed PMID: 5650164.

678 44. James A, Carroll N. Theoretic effects of mucus gland discharge on airway resistance in
679 asthma. *Chest*. 1995;107(3 Suppl):110S. Epub 1995/03/01. PubMed PMID: 7874986.

680 45. Kim V, Han MK, Vance GB, Make BJ, Newell JD, Hokanson JE, et al. The chronic bronchitic
681 phenotype of COPD: an analysis of the COPD Gene Study. *Chest*. 2011;140(3):626-33. Epub
682 2011/04/09. doi: 10.1378/chest.10-2948. PubMed PMID: 21474571; PubMed Central PMCID:
683 PMC3168856.

684 46. Lange P, Nyboe J, Appleyard M, Jensen G, Schnohr P. Relation of ventilatory impairment
685 and of chronic mucus hypersecretion to mortality from obstructive lung disease and from all
686 causes. *Thorax*. 1990;45(8):579-85. Epub 1990/08/01. PubMed PMID: 2402719; PubMed Central
687 PMCID: PMC462624.

688 47. Moreno RH, Hogg JC, Pare PD. Mechanics of airway narrowing. *Am Rev Respir Dis*.
689 1986;133(6):1171-80. Epub 1986/06/01. doi: 10.1164/arrd.1986.133.6.1171. PubMed PMID:
690 3717766.

691 48. Pelkonen M, Notkola IL, Nissinen A, Tukiainen H, Koskela H. Thirty-year cumulative
692 incidence of chronic bronchitis and COPD in relation to 30-year pulmonary function and 40-year
693 mortality: a follow-up in middle-aged rural men. *Chest*. 2006;130(4):1129-37. Epub 2006/10/13.
694 doi: 10.1378/chest.130.4.1129. PubMed PMID: 17035447.

695 49. Prescott E, Lange P, Vestbo J. Chronic mucus hypersecretion in COPD and death from
696 pulmonary infection. *Eur Respir J*. 1995;8(8):1333-8. Epub 1995/08/01. PubMed PMID: 7489800.

697 50. Rogers DF. Airway goblet cells: responsive and adaptable front-line defenders. *Eur Respir J*.
698 1994;7(9):1690-706. Epub 1994/09/01. PubMed PMID: 7995400.

699 51. Sherman CB, Xu X, Speizer FE, Ferris BG, Jr., Weiss ST, Dockery DW. Longitudinal lung
700 function decline in subjects with respiratory symptoms. *Am Rev Respir Dis*. 1992;146(4):855-9.
701 Epub 1992/10/11. doi: 10.1164/ajrccm/146.4.855. PubMed PMID: 1416410.

702 52. Shimura S, Andoh Y, Haraguchi M, Shirato K. Continuity of airway goblet cells and
703 intraluminal mucus in the airways of patients with bronchial asthma. *Eur Respir J*. 1996;9(7):1395-
704 401. Epub 1996/07/01. PubMed PMID: 8836649.

705 53. Speizer FE, Fay ME, Dockery DW, Ferris BG, Jr. Chronic obstructive pulmonary disease
706 mortality in six U.S. cities. *Am Rev Respir Dis*. 1989;140(3 Pt 2):S49-55. Epub 1989/09/01. doi:
707 10.1164/ajrccm/140.3_Pt_2.S49. PubMed PMID: 2782760.

708 54. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for
709 the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD
710 executive summary. *Am J Respir Crit Care Med*. 2013;187(4):347-65. Epub 2012/08/11. doi:
711 10.1164/rccm.201204-0596PP. PubMed PMID: 22878278.

712 55. Vestbo J, Prescott E, Lange P. Association of chronic mucus hypersecretion with FEV1
713 decline and chronic obstructive pulmonary disease morbidity. Copenhagen City Heart Study
714 Group. *Am J Respir Crit Care Med*. 1996;153(5):1530-5. Epub 1996/05/01. doi:
715 10.1164/ajrccm.153.5.8630597. PubMed PMID: 8630597.

716 56. Yohannes AM, Mullerova H, Hanania NA, Lavoie K, Tal-Singer R, Vestbo J, et al. Long-term
717 Course of Depression Trajectories in Patients With COPD: A 3-Year Follow-up Analysis of the
718 Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints Cohort. *Chest*.

719 2016;149(4):916-26. Epub 2016/02/03. doi: 10.1016/j.chest.2015.10.081. PubMed PMID:
720 26836938.

721 57. Newcomb DC, Boswell MG, Huckabee MM, Goleniewska K, Dulek DE, Reiss S, et al. IL-13
722 regulates Th17 secretion of IL-17A in an IL-10-dependent manner. *J Immunol.* 2012;188(3):1027-
723 35. Epub 2012/01/03. doi: 10.4049/jimmunol.1102216. PubMed PMID: 22210911; PubMed
724 Central PMCID: PMC3262924.

725 58. Newcomb DC, Peebles RS, Jr. Th17-mediated inflammation in asthma. *Curr Opin Immunol.*
726 2013;25(6):755-60. Epub 2013/09/17. doi: 10.1016/j.coi.2013.08.002. PubMed PMID: 24035139;
727 PubMed Central PMCID: PMC3855890.

728 59. Wynn TA, Barron L, Thompson RW, Madala SK, Wilson MS, Cheever AW, et al. Quantitative
729 assessment of macrophage functions in repair and fibrosis. *Curr Protoc Immunol.* 2011;Chapter
730 14:Unit14 22. Epub 2011/04/05. doi: 10.1002/0471142735.im1422s93. PubMed PMID: 21462164;
731 PubMed Central PMCID: PMC3109612.

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