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1 **Title: Comment on “Tumor-initiating cells establish an IL-33–TGF- β niche**
2 **signaling loop to promote cancer progression”**

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32 **Abstract:**

33 Taniguchi *et al.* (Research Article, 17 July 2020, eaay1813) claim that the cytokine IL-33 induces
34 accumulation of tumor-associated macrophages expressing the IgE receptor FcεRI. While these
35 findings hold great therapeutic promise, we provide evidence that the anti-FcεRI antibody used in
36 this study is not specific for FcεRI on macrophages, which raises concerns about the validity of
37 some of the conclusions.

38

39

40 **Main Text:**

41

42 The recent article by Taniguchi *et al.* (1) showed that IL-33 induces the accumulation of a subset
43 of tumor-associated macrophages promoting malignant progression of squamous cell carcinoma.
44 A major claim of the study is that this macrophage population is characterized by expression of
45 the high-affinity IgE receptor (FcεRI). However, concerns have been raised about the specificity
46 of the anti-mouse FcεRI antibody (clone MAR-1) used by the authors, especially the potential of
47 this clone to cross-react with IgG Fcγ receptors (FcγRs) (2).

48

49 FcεRI is highly expressed by mast cells and basophils (3). Indeed, we found that MAR-1 stains
50 peritoneal mast cells, as well as blood and spleen basophils in wild-type (WT) mice, but not in
51 mice deficient for FcεRI (mFcεRI^{def} mice) (**Fig. 1, A-C**). In stark contrast, MAR-1 stains
52 peritoneal and spleen macrophages equally well in both WT and mFcεRI^{def} mice (**Fig. 1, D and**
53 **E**). These results indicate that in naïve mice, MAR-1 specifically recognizes FcεRI on mast cells
54 and basophils, but labels macrophages in a FcεRI-independent manner.

55

56 In their article, Taniguchi *et al.* proposed that IL-33 induces expression of FcεRI in bone marrow-
57 derived macrophages (1). We repeated these experiments using bone marrow cells from WT,
58 mFcεRI^{def}, and mFcγR^{null} mice, which lack all mouse FcγRs (4). We confirmed that IL-33-induced
59 macrophages derived from WT bone marrow were stained with MAR-1 (**Fig. 1F**). However, bone
60 marrow-derived macrophages from mFcεRI^{def} mice were stained to the same degree, further
61 corroborating that MAR-1 staining of macrophages is not FcεRI-specific (**Fig. 1F**). Tang *et al.*

62 reported potential cross-reaction of MAR-1 with the FcγRs I and IV (2). In keeping with this, we
63 found that IL-33-induced bone marrow-derived macrophages do express FcγRI and FcγRIV, and
64 critically, that MAR-1 does not stain macrophages derived from mFcγR^{null} bone marrow (**Fig. 1F**).
65 Altogether, these results indicate that the MAR-1 staining observed in IL-33-induced bone-marrow
66 derived macrophages relies solely on FcγR expression, and that these cells do not express FcεRI.

67

68 Finally, Taniguchi *et al.* purified ‘FcεRI⁺’ and ‘FcεRI^{neg}’ macrophages from squamous cell
69 carcinoma based on MAR-1 staining and analyzed their transcriptomes by RNA-seq (accession
70 number GSE151783). In an attempt to clarify the identity of these populations in an unbiased,
71 genome-wide manner, we compared their gene expression profiles to publicly available data from
72 several highly purified immune cell populations (5), using the CIBERSORT method (6). This
73 approach showed that both populations largely align to the macrophage/monocyte lineage
74 signatures (**Fig. 1G**), confirming that the ‘FcεRI⁺’ cells are indeed macrophages. Even though cells
75 were sorted based on MAR-1 staining, we detected virtually no *Fcer1a* mRNA signal for both
76 MAR-1^{high} and MAR-1^{low} population in the authors’ dataset (**Fig. 1H**). By contrast, mRNA was
77 abundant for all four FcγRs (FcγRI, IIB, III and IV) (**Fig. 1H**).

78

79 Based on these data, we conclude that the tumor-associated macrophage population described by
80 Taniguchi *et al.* is unlikely to express FcεRI. To highlight the usefulness of MAR-1 labeling for
81 the identification of these cells, but avoid confusion about functional implications, we suggest
82 referring to them as ‘MAR-1^{high} macrophages’ instead (a similar nomenclature is already used for
83 monocyte-derived dendritic cells (7)). Although our in vitro data suggest that MAR-1 exclusively

84 binds FcγRs on macrophages (**Fig. 1F**), the lack of significant differences in FcγR mRNA between
 85 MAR-1^{high} and MAR-1^{low} tumor-associated macrophages observed by RNAseq (**Fig. 1H**) suggests
 86 that additional surface molecules might be involved in MAR-1 labeling in vivo. It will be important
 87 to characterize further which surface molecules are recognized by MAR-1 in tumor-associated
 88 macrophages, and whether these molecules represent potential targets for the development of
 89 cancer treatments.

90

91 **References and Notes:**

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 109 place of the mouse *Fcer1a* gene, thereby abrogating expression of mouse FcεRIα.
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 112 10. ImmGen (<http://www.immgen.org>) Microarray Phase 2 data were downloaded from GEO
 113 and normalized using Robust Multi-array Average (RMA) method. Six highly purified cell
 114 populations (basophils, innate lymphoid cells [ILC], macrophages, mast cells, monocytes
 115 and neutrophils) were selected and CIBERSORT was used to generate gene signatures for
 116 each population. With these gene signatures, CIBERSORT was run to de-convolute the
 117 MAR-1⁺ and MAR-1^{neg} cell populations purified by Taniguchi *et al* (GSE151783), and
 118 generate fraction scores which were then visualized in stacked bar chart using R.
 119

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134

135 **Competing interests:**

136 The authors declare that they have no competing interests.

137

138 **Author contributions:**

139 Experimental design, J.B.J.K, W.P.M.W, L.G, N.G, R.G, J.C. and L.L.R; Investigation, J.B.J.K,
140 W.P.M.W, J.S., E.C., A.M, E.M, L.G., N.G, R.G., J.C. and L.L.R; Formal analysis, J.B.J.K,
141 W.P.M.W, A.M, E.M, R.G, J.C. and L.L.R; Provided bone marrow cells from mFcyR^{null} mice:

142 P.B; Writing (original draft), J.B.J.K, W.P.M.W and L.L.R; Writing (review and editing), all
143 authors.

144 **Figure Legend:**

145

146 **Figure 1. Evidence that various macrophage populations, including IL-33-induced**
147 **macrophages and tumor-associated macrophages, do not express FcεRI.**

148 (A–E) Quantification (mean fluorescence intensity [MFI]) of staining with MAR-1 or an isotype
149 control antibody (Iso; Armenian hamster IgG) on peritoneal mast cells (F4/80⁻, CD115⁻, KIT⁺)
150 (A), blood (B) and spleen (C) basophils (CD45^{int}, CD131⁺), spleen macrophages (MΦ; Ly6G⁻,
151 F4/80⁺) (D), and peritoneal macrophages (F4/80⁺, CD115⁺) (E) from wild-type (WT) and
152 mFcεRI^{def} mice (8). Results in A–E show values from individual mice; bars indicate means ± SEM
153 pooled from three (B and C) or four (A, D, and E) independent experiments. **, $P < 0.01$; ***, P
154 < 0.001 by Mann–Whitney U test. N.S. not significant ($P > 0.05$). (F) Analysis of staining with
155 MAR-1, an anti-FcγRI antibody (clone REA286), or an anti-FcγRIV antibody (clone 9E9) in IL-
156 33-induced macrophages derived from KIT⁺ bone marrow cells from WT, mFcεRI^{def}, and
157 mFcγR^{null} mice following the protocol described by Taniguchi *et al.* (1) and Wilkinson *et al.* (9).
158 Results are representative of three independent experiments. FMO: fluorescence minus one
159 control. (G and H) Raw RNAseq data for MAR-1^{high} and MAR-1^{low} tumor-associated
160 macrophages from Taniguchi *et al.* (1) were downloaded from GEO (GSE151783) and normalized
161 to count per million (CPM) using the edgeR R package. (G) Reference gene expression signatures
162 specific for different innate immune cell lineages (basophils, innate lymphoid cells [ILCs],
163 macrophages, mast cells, monocytes, and neutrophils) were generated using publicly available
164 ImmGen datasets from highly purified cell populations. The relative abundance of these reference
165 signatures was then quantified in the gene expression data from MAR-1^{high} and MAR-1^{low} tumor-
166 associated macrophages using CIBERSORT, to estimate the likelihood of these cells belonging to
167 the macrophage lineage (6, 10). (H) *Fcer1a*, *Fcgr1*, *Fcgr2b*, *Fcgr3*, and *Fcgr4* RNA-seq data
168 (CPM values) for MAR-1^{high} and MAR-1^{low} tumor-associated macrophages.

