Comment on "Tumor-initiating cells establish an IL-33–TGF-β niche signaling loop to promote cancer progression"

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Title: Comment on "Tumor-initiating cells establish an IL-33–TGF-β niche signaling loop to promote cancer progression"

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Abstract:

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

Main Text:

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

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

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

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

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

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

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

References and Notes:

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8. We used hFcεRIα^{KI} mice from Genoway, in which the human *Fcer1a* gene is inserted in place of the mouse *Fcer1a* gene, thereby abrogating expression of mouse FcεRIα.
9. A. C. Wilkinson *et al.*, Long-term ex vivo haematopoietic-stem-cell expansion allows nonconditioned transplantation. *Nature* **571**, 117-121 (2019).
10. ImmGen (http://www.immgen.org) Microarray Phase 2 data were downloaded from GEO and normalized using Robust Multi-array Average (RMA) method. Six highly purified cell populations (basophils, innate lymphoid cells [ILC], macrophages, mast cells, monocytes and neutrophils) were selected and CIBERSORT was used to generate gene signatures for each population. With these gene signatures, CIBERSORT was run to de-convolute the MAR-1^{+} and MAR-1^{neg} cell populations purified by Taniguchi *et al* (GSE151783), and generate fraction scores which were then visualized in stacked bar chart using R.

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Competing interests:

The authors declare that they have no competing interests.

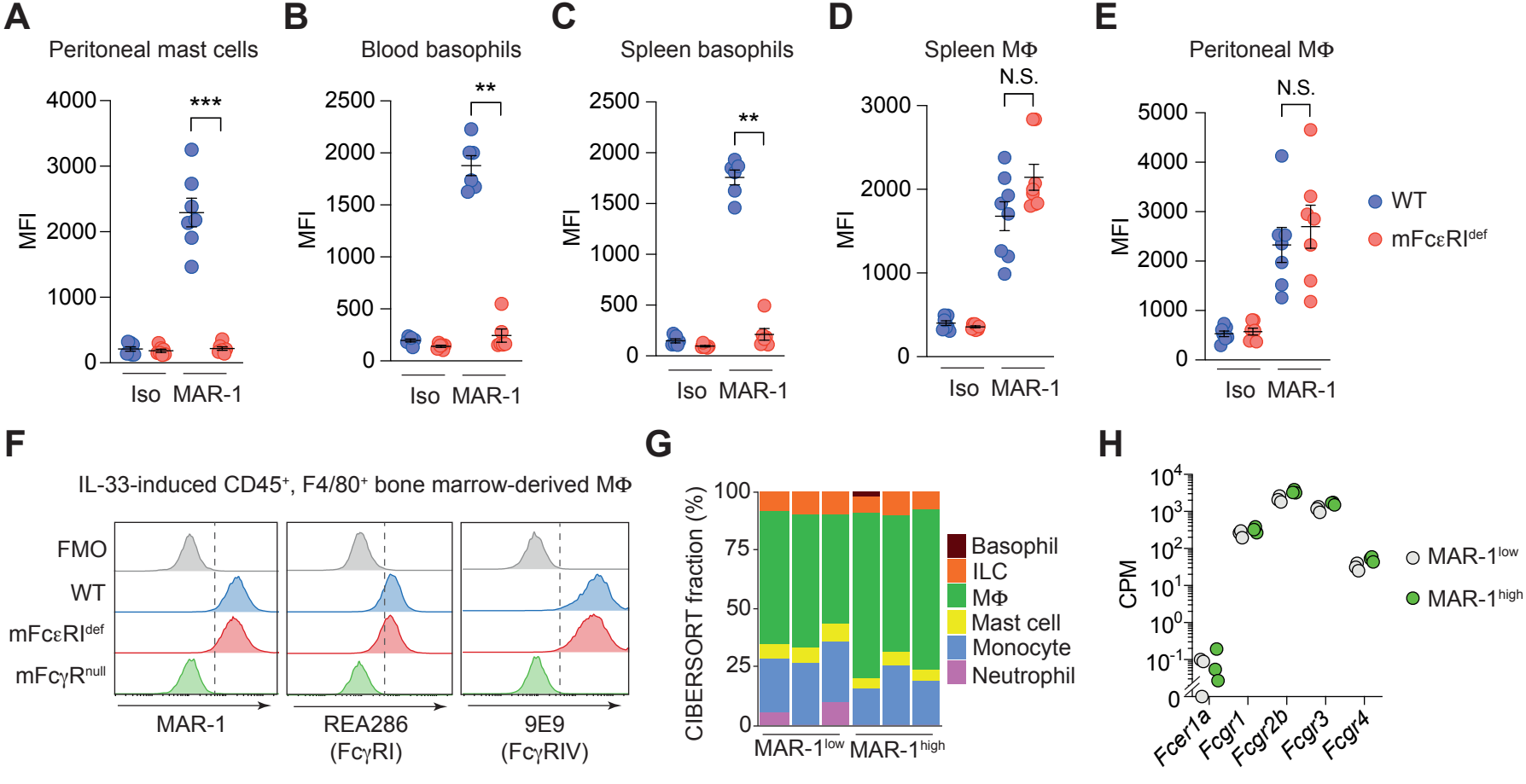
Author contributions:

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, 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, W.P.M.W, A.M, E.M, R.G, J.C. and L.L.R; Provided bone marrow cells from mFcγR^{null} mice:

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

Figure Legend:

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


A
Peritoneal mast cells
MFI
4000
3000
2000
1000
0
***
Iso
MAR-1
B
Blood basophils
2500
2000
1500
1000
500
0
**
C
Spleen basophils
D
Spleen MΦ
3000
N.S.
E
Peritoneal MΦ
5000
WT
mFcεRI^def
F
IL-33-induced CD45+, F4/80+ bone marrow-derived MΦ
FMO
WT
mFcεRI^def
mFcγR^null
MAR-1
REA286
(FcγRI)
9E9
(FcγRIV)
G
CIBERSORT fraction (%)
100
75
50
25
0
MAR-1^low
MAR-1^high
Basophil
ILC
MΦ
Mast cell
Monocyte
Neutrophil
H
CPM
10^4
10^3
10^2
10^1
10^0
10^-1
0
Fcer1a
Fcgr1
Fcgr2b
Fcgr3
Fcgr4
MAR-1^low
MAR-1^high