Questioning whether IgM Fc receptor (FcµR) is expressed by innate immune cells

Christopher M. Skopnik¹, René Riedel¹, Richard K. Addo¹, Gitta Anne Heinz¹, Frederik Heinrich¹, Kazuhiro Honjo², Pawel Durek¹, Philipp Enghard³, Mir-Farzin Mashreghi¹, Andreas Radbruch¹ & Hiromi Kubagawa¹✉

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Fc receptors (FcRs) are effector molecules that permit binding to antibodies. Recently, Kubli et al. reported that the FcR for IgM (FcµR) was expressed by non-B cells, i.e., tumor-associated mononuclear phagocytes (TMPs), and was involved in anti-tumor immunity¹. Here, we have examined whether FcµR is indeed expressed by these cells. Our results do not support their FcµR expression.

Antibody or immunoglobulin (Ig), a key player in humoral immunity, has dual binding activities: to antigens via its amino-terminal variable regions (called Fab) and to effector molecules via its carboxy-terminal constant region (called Fc). One such effector molecule is a family of cell surface FcRs. FcRs for switched Igs are expressed by many different immune cells, including myeloid cells, and function as central mediators coupling innate and adaptive immune responses. FcµR, identified in 2009, is the newest member of the FcR family. Conflicting views exist especially with regard to its cellular distribution in mice: B cells only versus B, myeloid and T cells (see a recent review²). Kubli et al. have focused on the function of FcµR in non-B cell populations using his FcµR-deficient (KO) mice. They recently reported that FcµR negatively regulated the anti-tumor activity of TMPs (CD45⁺ CD11b⁺ MerTk⁺ CD64⁺) in a melanoma mouse model¹. FcµR KO mice had increased numbers of TMPs, reduced tumor size and enhanced survival compared to wild type (WT) controls. Single-cell RNA sequence (scRNAseq) analyses of the TMPs from FcµR KO and WT mice revealed a unique TMP subset with enhanced antigen processing/presenting properties in the mutant mice. On the other hand, we and others have focused on FcµR function in B cells², for the simple reason that we have never found expression of FcµR by non-B cells, including myeloid cells, using immunofluorescence analysis with receptor-specific monoclonal antibodies (mAbs) and sensitive reverse transcription polymerase chain reaction assays³,⁴. In this report, we show that FcµR is not expressed by TMPs based on our examination of the scRNAseq data GSE130287 of Kubli et al.¹ using the R software (version 4.1). We also show our scRNAseq data GSE140133 from splenic IgG memory B cells in C57BL/6 mice⁵ for comparative purposes and provide comments on their data.

As shown in the gene detection histogram based on raw data (Fig. 1a), nearly all of the TMPs (6352 FcµR WT and 8000 FcµR KO cells) had no FcµR transcript reads [i.e., zero unique molecular identifier (UMI)]. Only four FcµR WT cells (0.06%) had 4 (1) or 1 (3) UMIs per cell and six FcµR KO cells (0.08%) had 1 UMI per cell. Notably, the one WT cell with four UMIs also contained transcripts of B cell-specific genes (e.g., Cd79a) at similar UMI counts. Our controls included a house-keeping gene (Gapdh) and randomly picked, high (Cd74 and Cd68) and low (Igam and Cd86) abundance genes. The reads variably ranged: 0 to >300 for Gapdh, 0 to >100 for high and 0 to >15 for low abundance genes. To rule out the possibility that FcµR transcripts are somehow undetectable in scRNAseq assessments as is often seen with certain genes, e.g., cytokines, we performed scRNAseq analysis with splenic IgG memory B cells from WT mice (Fig. 1b). Unlike in TMPs, FcµR transcripts were easily detectable in ~75% of the IgG memory B cells at 1 to ~65 UMIs per cell. This is more evident by density curves (Fig. 1c) after normalization on the basis of sequencing depth. One of the high abundance genes Cd74 in TMPs and Gapdh are also highly or clearly expressed by IgG memory B cells. Cd68 and Igam transcripts were present in TMPs but not in IgG B cells. The expression of Cd86 was comparable in both cell types. Kubli et al. emphasized FcµR expression by non-B cells in the introduction of the paper, citing several references including theirs, but they did not describe any FcµR transcript results from scRNAseq analysis in the text¹. Collectively, according to our analysis of their TMP scRNAseq data, we can conclude that there is no evidence for FcµR gene expression by mononuclear phagocytes infiltrating around tumors or TMPs.

Then, how can we explain the differences observed in FcµR-negative TMP-mediated anti-tumor responses and dendritic cell functions between FcµR KO and WT mice as described in the paper? Several considerations or potential causes are noteworthy but none of them are definitive. (i) As compared to other FcµR...
Fig. 1 Gene detection histogram. a, b Raw values for the number of cells and the number of transcript reads (or unique molecular identifier; UMI) of the indicated genes in tumor-associated mononuclear phagocytes (TMPs) from C57BL/6 Fcmr WT (upper) and KO (lower) mice (a) and IgG memory B cells from C57BL/6 WT spleen (b), are plotted on y-axes and x-axes, respectively. c Density curves of normalized UMIs for Fcmr transcripts in TMPs from Fcmr WT and KO mice and from WT splenic IgG B cells. The TMP data were derived from GSE130287 and the IgG B cell data from our scRNAseq analysis GSE140133. Note different scales of the x-axis for each gene.
KO mice including ours, the FcμR KO mice of Kubli et al. are unique in extensive targeting of exon 2–8 (~10 kb) and retention of the Neo gene in the mouse genome. This may account for discrepancies in reported phenotypes among mutant mice. In fact, a difference in granulocyte function between their and our mutant (targeting exon 2–4) mice was noted. Production of reactive oxygen species (ROS) was higher in their FcμR KO granulocytes than WT controls upon stimulation with fMLP in unique in extensive targeting of exon 2 granulocytes. Another unexpected finding is the restricted distribution of FcμR orthologues to mammals, despite the phylogenetically broad distribution of IgM from jawed vertebrate onward. These findings suggest that lymphocyte-specific FcμR must have distinct functions compared to broadly distributed FcRs for switched Ig isotypes. In summary, based on our analysis of the scRNAseq data on TPMs, we demonstrate the absence of FcμR transcripts in such infiltrating monocytoid cells around tumor tissues. Since the authors elegantly described the potential tumor immunity by such TPMs through FcμR without sufficiently backing their FcμR expression results at the single cell level, we would call the readers’ attention to this discrepancy when reading this paper.

Methods

Single cell analyses. The scRNAseq data of TPMs (GSE130287) from FcμR WT and KO C57BL/6 mice5 and splenic IgG memory B cells (GSE140133) from antigen-immunized WT C57BL/6 mice6, which are publicly available (see their repositories below), were reexamined as follows. R package Seurat v4.0.511 was used to read deposited data sets. Raw count matrices were subject to quality control based on: (i) the number of cells where a gene was detected, (ii) the number of genes detected per cell, and (iii) the frequency of UMIs associated with mitochondrial genes. The final filtered matrices contained 16,112 genes and 14,352 cells (6352 FcμR WT and 8000 FcμR KO mice) for TPMs and 13,072 genes and 5436 cells for IgG B cells. Seurat LogNormalize-method with default settings was used to normalize UMI counts.

Analysis of ROS production. For ROS production, blood was collected from the submandibular vein of FcμR WT and KO C57BL/6 female mice of 8–12 weeks by needle puncture in heparinized microfuge tubes. Twenty microliter of blood were mixed with 180 µl of 10 µM dihydrodorhodamine 123 (DHR123; DHR123; Markser Gene Technologies) in DMEM/2% FCS in 96-well flat-bottom plates. After 30 min under 5% CO2 at 37 °C, cells were washed with FACS buffer (PBS/2% FCS/0.1% NaN3) and stained with PE-labeled Gr-1 mAb (clone M1/70; eBioscience) at 0.67 µg/ml on ice for 20 min, before lysis of erythrocytes with 1 ml of Ficoll/Lysis buffer (PBS/1% formalin/0.1% saponin) on ice for 10 min. After washing, cells were resuspended in FACS buffer and granulocytes were first gated by high FSC and high SSC characteristics and then by positive for both Gr-1 and CD11b by Accuri C6 Flow Cytometer (BD). DHR123+ Gr-1+ CD11b+ cells were defined as FcμR-producing granulocytes. Approximately 50,000–70,000 cell events were acquired and analyzed with FlowJo software (BD). On average 5% of total blood nucleated cells were granulocytes for both groups of mice. Data comparison was performed by unpaired Student t-test (GraphPad Prism 9) and a two-tailed P value of <0.05 was defined as statistically significant. All studies involving animals were conducted with and after approval of the Institute for Gesundheit und Soziales (Lageso) and University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC-09195).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The authors declare that all data supporting the findings of this study are available within this article or from the corresponding author upon request. Single cell RNA-sequencing data of TPMs and IgG memory B cells have been deposited in the Gene Expression Omnibus (GEO) repository by the original authors, Kubli et al. and Riedel et al. Accession codes are GSE130287 and GSE140133, respectively.
Code availability
The R code to reproduce Fig. 1 is deposited at the public GitHub repository https://github.com/Close-your-eyes/NCOMMS-19-25487A.

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Author contributions
C.M.S. and P.E. analyzed publicly available TMP scRNAseq data (GSE130287) (Fig. 1a, c); R.R., R.K.A., G.A.H., F.H., P.D., M.F.M., and A.R. performed IgG memory B cell scRNAseq (GSE140133) (Fig. 1b, c); K.H. analyzed granulocyte function (Fig. 2); H.K. wrote this Matters Arising; and all authors reviewed it and approved its final form.

Competing interests
The authors declare no competing interests.

Additional information
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Correspondence and requests for materials should be addressed to Hiromi Kubagawa.

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