Antibody-dependent enhancement (ADE) in Covid-19 physiopathology, a cross-model single-cell RNA-seq perspective

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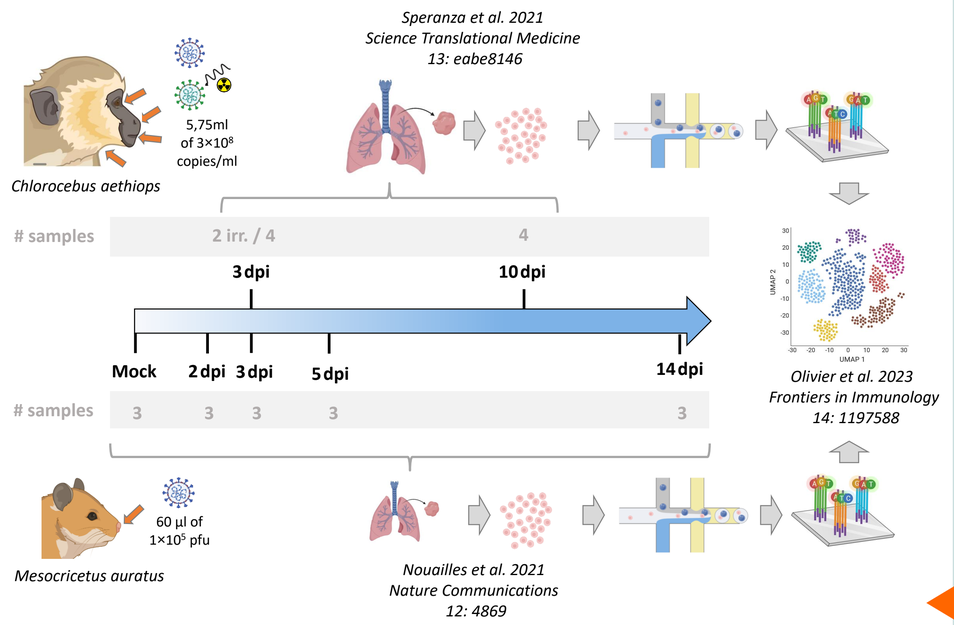
BACKGROUND **Cytokine storm** is considered a driving factor of **COVID-19 severity**. However, the triggering and resolution of this cytokine production as well as the link between this phenomenon and infected cells is still poorly understood. Considering that **M1 macrophages** are specialized for pathogen killing and secrete **pro-inflammatory cytokines** as well as **chemokines** upon Ifn-γ activation, an assessment of their contributions in cytokine storms is necessary. Moreover, considering that **Fc receptors for immunoglobulin G (FcγR)** expression was found to be stimulated by Ifn-γ in monocytes and macrophages and that FcγR markedly affect antigen uptake in macrophages, exploring the potential link between **FcγR-mediated antibody-dependent infections of M1 macrophages** and the **surge of cytokine storms caused by SARS-CoV-2 infections** constitutes a promising avenue of research to explain the phenomenon of **antibody-dependent enhancement** (**ADE**).

METHODS In this work, previously published **scRNA-seq** prepared from lungs of **Syrian hamsters** and **African green monkeys** (**AGM**) were **reanalyzed in a common uniform manifold approximation and projection (UMAP)** to **follow the cellular expression of the virus as well as immune related genes during the infection in both species**. To this end, **10 AGM** and **15 hamster scRNA-seq runs,** generated as shown on the **right-hand side figure**, were further characterized using the latest reference transcriptomes supplemented with the **positive and negative genomic sequences of SARS-CoV-2** as well as the **constant sequences of BCR and TCR** of both species.
The resulting count matrices from hamsters and AGM, generated with Salmon-Alevin, were integrated using an **ortholog correspondence table generated with tBLASTx** and the SCTransform workflow of Seurat R package. No prior filtering was applied to count matrices to keep **apoptotic cells** and **biologically relevant duplets**. Cells clustered as **apoptotic cells** were further characterized using **univariate UMAP analyses**, where scRNA-seq runs from the same time point and species were analyzed separately to trace back their original cell type. **Cell types were determined** according to differentially expressed genes and cell type markers mentioned in the literature.



RESULTS We found that 1) **M1 macrophages and pneumocytes are the main contributors of viral transcripts** in both species (**Figure 1**), 2) **viral read-bearing cells show dying cells/apoptotic phenotype whatever the cell type** (**Figure 2**), 3) **B cell activation** was detected **from the earliest time points of infection** (**Figure 3**), 4**) antibody isotypes overexpressed** during and after infection are **known to bind FcγR**, **the latter being upregulated in viral read bearing macrophages** (**Figure 3 and 4**), 5) **FcγR better correlated with infected cells** than known SARS-CoV-2 entry receptors Ace2, Nrp1 and Axl in both species (**Figure 4**), 6) **type 2 interferon response** was observed in both species together with the **induction of FcγR** (**Figures 3** and **4**), 7) **chemokines peak together with inflammation** at the **tipping point of infection** (**see publication**).

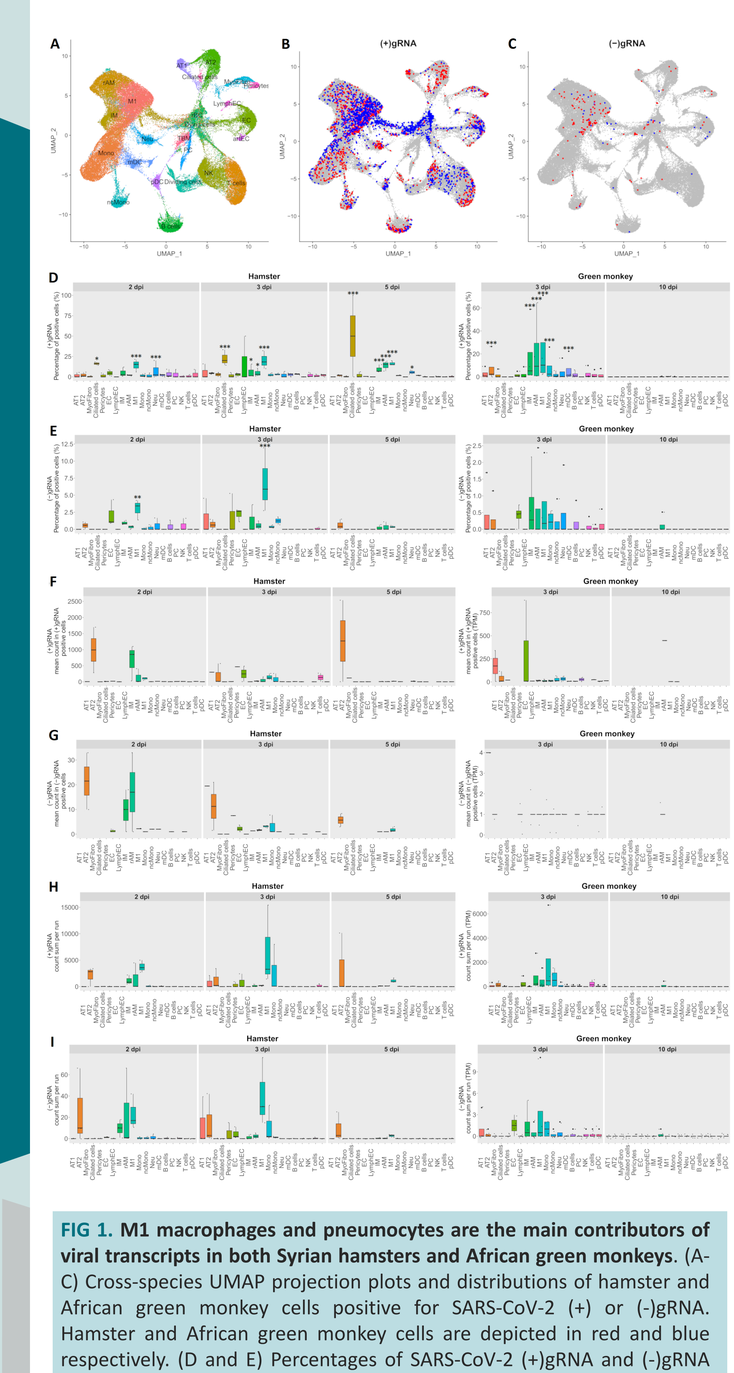


FIG 1. M1 macrophages and pneumocytes are the main contributors of viral transcripts in both Syrian hamsters and African green monkeys. (A-C) Cross-species UMAP projection plots and distributions of hamster and African green monkey cells positive for SARS-CoV-2 (+) or (-)gRNA. Hamster and African green monkey cells are depicted in red and blue respectively. (D and E) Percentages of SARS-CoV-2 (+)gRNA and (-)gRNA respectively for hamsters at 2, 3, and 5 dpi and AGM at 3 and 10 dpi. (F and G) Mean numbers of SARS-CoV-2 (+)gRNA and (-)gRNA counts respectively for the same experimental modalities. (H and I) Sums of SARS-CoV-2 (+)gRNA and (-)gRNA counts respectively for the same experimental modalities.
*: p <= 0.05, **: p <= 0.01, .***: p <= 0.001

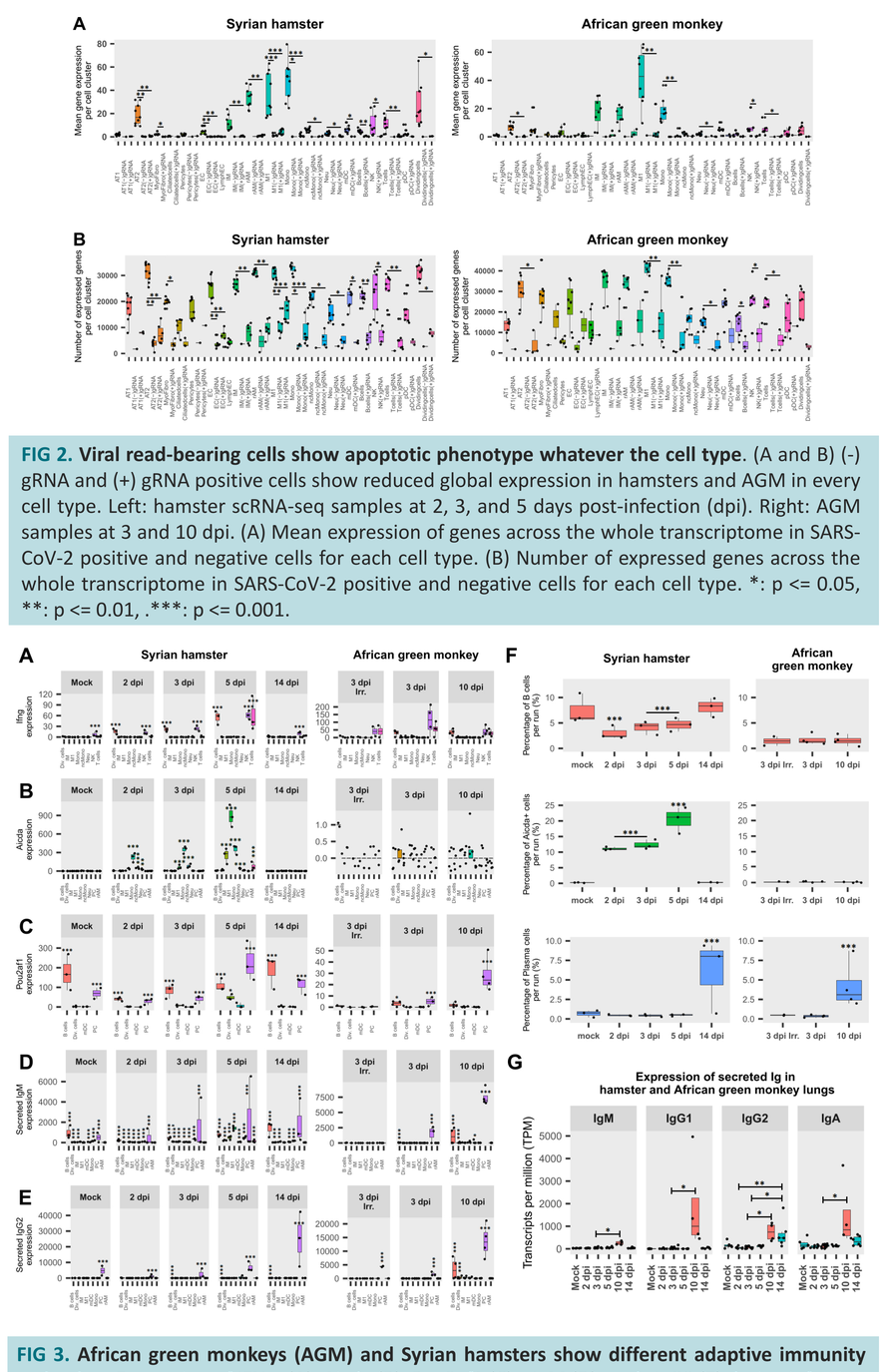


FIG 2. Viral read-bearing cells show apoptotic phenotype whatever the cell type. (A and B) (-) gRNA and (+) gRNA positive cells show reduced global expression in hamsters and AGM in every cell type. Left: hamster scRNA-seq samples at 2, 3, and 5 days post-infection (dpi). Right: AGM samples at 3 and 10 dpi. (A) Mean expression of genes across the whole transcriptome in SARS-CoV-2 positive and negative cells for each cell type. (B) Number of expressed genes across the whole transcriptome in SARS-CoV-2 positive and negative cells for each cell type. *: p <= 0.05, **: p <= 0.01, .***: p <= 0.001.

FIG 3. African green monkeys (AGM) and Syrian hamsters show different adaptive immunity activation. Expression of IFN-γ (A), Aicda (B), Pou2af1 (C), sIgM (D), and sIgG2 (E) during infection in Syrian hamsters and African green monkeys according to lung pseudobulk scRNA-seq and expressed in the sum of counts. (F) Percentage of Aicda-negative B cells, Aicda-positive cells, and plasma cells in each scRNA-seq run for each modality. (G) Expression of secreted immunoglobulins M, G1, G2, and A during SARS-CoV-2 infection in AGM (red) and hamster (green) lungs. *: p <= 0.05, **: p <= 0.01, .***: p <= 0.001.

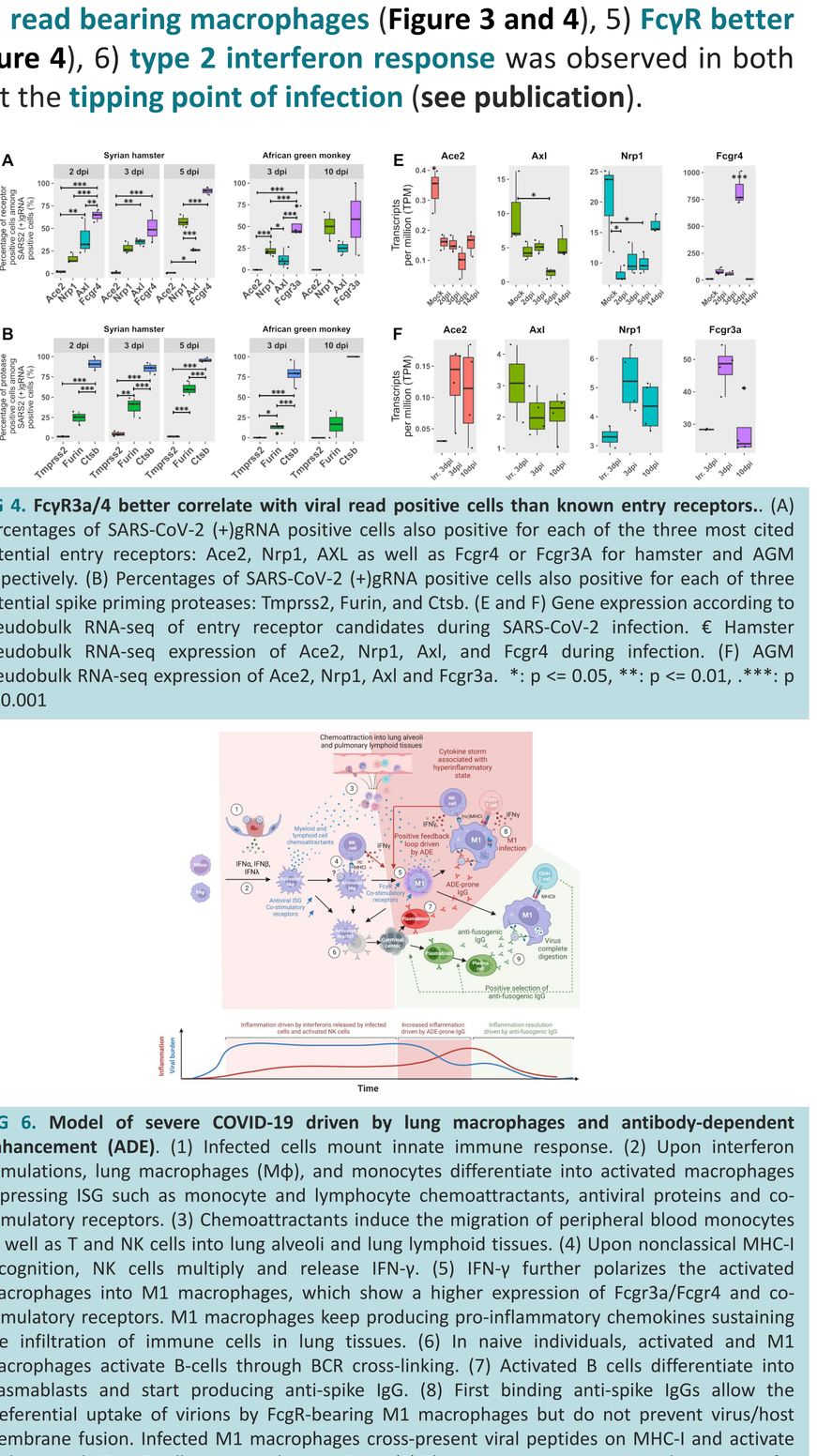


FIG 4. FcγR3a/4 better correlate with viral read positive cells than known entry receptors.. (A) Percentages of SARS-CoV-2 (+)gRNA positive cells also positive for each of the three most cited potential entry receptors: Ace2, Nrp1, AXL as well as Fcgr4 or Fcgr3A for hamster and AGM respectively. (B) Percentages of SARS-CoV-2 (+)gRNA positive cells also positive for each of three potential spike priming proteases: Tmprss2, Furin, and Ctsb. (E and F) Gene expression according to pseudobulk RNA-seq of entry receptor candidates during SARS-CoV-2 infection. € Hamster pseudobulk RNA-seq expression of Ace2, Nrp1, Axl, and Fcgr4 during infection. (F) AGM pseudobulk RNA-seq expression of Ace2, Nrp1, Axl and Fcgr3a. *: p <= 0.05, **: p <= 0.01, .***: p <= 0.001

FIG 6. Model of severe COVID-19 driven by lung macrophages and antibody-dependent enhancement (ADE). (1) Infected cells mount innate immune response. (2) Upon interferon stimulations, lung macrophages (Mϕ), and monocytes differentiate into activated macrophages expressing ISG such as monocyte and lymphocyte chemoattractants, antiviral proteins and co-stimulatory receptors. (3) Chemoattractants induce the migration of peripheral blood monocytes as well as T and NK cells into lung alveoli and lung lymphoid tissues. (4) Upon nonclassical MHC-I recognition, NK cells multiply and release IFN-γ. (5) IFN-γ further polarizes the activated macrophages into M1 macrophages, which show a higher expression of Fcgr3a/Fcgr4 and co-stimulatory receptors. M1 macrophages keep producing pro-inflammatory chemokines sustaining the infiltration of immune cells in lung tissues. (6) In naive individuals, activated and M1 macrophages activate B-cells through BCR cross-linking. (7) Activated B cells differentiate into plasmablasts and start producing anti-spike IgG. (8) First binding anti-spike IgGs allow the preferential uptake of virions by FcgR-bearing M1 macrophages but do not prevent virus/host membrane fusion. Infected M1 macrophages cross-present viral peptides on MHC-I and activate both NK and CD8+ T cells in turn releasing IFN-γ. (9) The continuous activation and mutation of B cells generate the first anti-fusogenic anti-spike antibodies. The latter allow the complete digestion of viral proteins and the presentation of viral peptides on MHC-II. This in turn allows the activation of CD4+ T cells, which promote the selection and differentiation of anti-fusogenic IgG-producing plasmocytes. This figure together with the figure of the method section were created with BioRender.com.

CONCLUSIONS Our results confirm the recent experimental demonstration that SARS-CoV-2 infects macrophages and indicate that **COVID-19 severity increases through a positive feedback loop** involving: 1) **interferon polarized, pro-inflammatory cytokine-producing M1 macrophages** cross-presenting viral peptides on MHCI upon **antibody-dependent infection** and 2) **chemoattracted and proliferating NK and T cells producing Ifn-γ upon MHCI ligation**. We propose a model in which this positive feedback loop of inflammation driven by fusogenic antibodies constitutes the basic mechanism of the **antibody-dependent enhancement** (**ADE**) phenomenon.