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Previews

Interfer(on)ing with Zika virus

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In this issue of *Neuron*, Bulstrode et al.[1] demonstrate that glioblastoma slice cultures, unlike neural progenitors, are refractory to Zika virus infection. The anti-infective mechanism is myeloid-lineage cell-secreted interferon beta. These studies have implications for therapeutics in both glioblastoma and Zika virus infections.

During the Zika virus (ZIKV) epidemic in 2015–2016, approximately 1.5 million people were infected in Brazil alone. Infection of pregnant women resulted in a Zika congenital syndrome, which included a spectrum of neurodevelopmental delay with the most severely affected displaying overt microcephaly.[2] Later studies showed the virus could infect a neural progenitor population characterized by expression of SRY-box transcription factor 2 (SOX2).[3] These findings were of particular interest to brain cancer researchers, as SOX2 has been associated with glioblastoma (GBM) progression and to glioma stem cell (GSC) maintenance.[4,5] Taking advantage of this finding, studies were performed demonstrating that the hard-to-kill SOX2-positive glioblastoma stem cells could be infected by ZIKV, prolonging mouse survival in preclinical studies.[6,7]

Surprisingly, Bulstrode et al.[1] found major differences in productive ZIKV infection when comparing human developing brain (HDB) and GBM slice cultures, despite both having comparable cell populations expressing SOX2. HDB samples showed persistent infection 72 h post exposure, while GBM slices remained refractory. Similar results were observed 7 days post exposure using quantitative real-time PCR measuring viral copy number. Importantly, cell lines derived from adult GBMs were susceptible to ZIKV infection, in accordance with previous reports.[6,8] These findings suggested that the differential response to viral exposure was a consequence of microenvironmental factors and not an intrinsic characteristic of SOX2-expressing cells. To address this, the authors prepared primary HDB and GBM single-cell suspensions containing multiple cellular populations. Again, samples from developing brain were susceptible to ZIKV infection, whereas all 20 GBM samples showed much lower infection rates and needed longer exposure (72 h vs. 48 h) to be detectable. To investigate the effects of infection, single-cell suspensions were infected with a labeled ZIKV and sorted to evaluate gene expression in positive and negative fractions from GBMs they had identified as highly resistant (HR) or moderately resistant (MR) to infection using bulk RNA sequencing. A very strong immune signature composed of human leukocyte antigen (HLA), CD74, IBA1, CD45, and CD11b was enriched in the HR samples compared with MR ones. Using deconvolution analysis, the authors detected a glioma myeloid signature in HR GBMs. Moreover, staining with markers of myeloid populations IBA1 and RUNX1 confirmed a higher abundance of myeloid cells in HR compared with MR samples. Finally, comparison of positive (infected) and negative (non-infected) fractions revealed enrichment of pro-inflammatory cytokines including IFNB1, IFNL1, and CCL5, which were absent in HDB infected tissue slices (Figure 1).

Recent years have seen an increasing number of reports underscoring the importance of the tumor microenvironment (TME). There is a complex interaction between tumor cells, non-transformed neurons, and glia and immune cells. A large body of literature exists that supports the hypothesis that GBM cells help to create an immunosuppressive microenvironment, inhibiting the influx of potentially tumor-suppressive T cells and promoting the local macrophages and microglia—which can make up a large percentage of the tumor mass—to take on an immunosuppressive phenotype.[9] The presence of a myeloid signature that could prevent an effective infection in GBM cells is a novel finding and expands our knowledge of tumor-immune interactions.

The implication of myeloid cells in tumor resistance to ZIKV raised several questions to the authors, including whether a myeloid component is necessary and sufficient and whether the mechanism is through direct cell contact or via the release of immune modulatory factors. To answer the first question, magnetic antibody-based cell sorting (MACS) was used to separate CD11b-positive (myeloid) and -negative fractions from primary GBM samples. CD11b-depleted-GBM cultures had higher copies of the virus compared to the unsorted control. To test if their presence alone is sufficient to provide protection, CD11b+ cells were cocultured with GBM lines that had shown higher rates of infection. Addition of the myeloid compartment in a 1:2 ratio resulted in a greater than 10-fold reduction of virus copies; similar results were observed when using permissive HDB lines. These data demonstrate that CD11b+ cells inhibit the ZIKV infection. To answer the question of whether the inhibitory effect of myeloid cells requires direct cell contact or whether it is mediated by secreted factors, the authors obtained conditioned media from CD11b+ and CD11b− cells that were then used for culturing with ZIKV infection-sensitive cells and for cytokine secretome analysis. Conditioned media from the CD11b+ fraction was able to prevent high numbers of ZIKV copies in previously permissive lines. This conditioned media was also highly enriched in interferon (IFN)-β, CXCL8, tumor

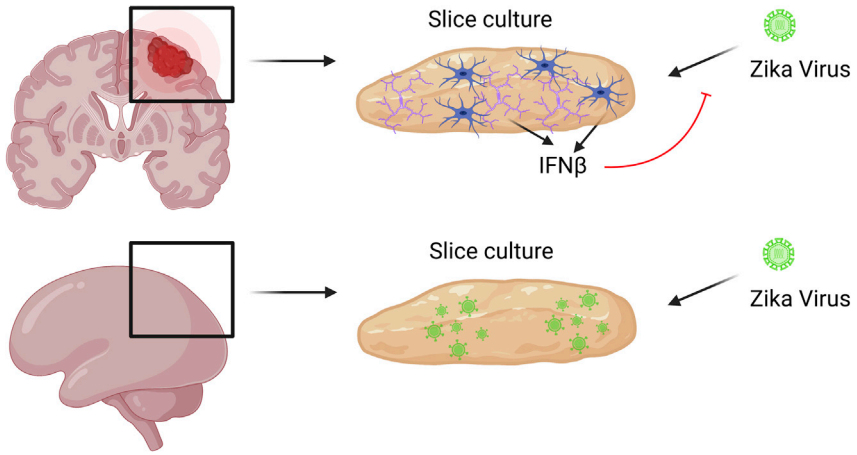

Figure 1. Slice cultures from glioblastoma contain CD11b+ cells (microglia and macrophages) that produce IFN-β and prevent Zika infection of progenitor cells
Human developing brain is infection-permissive as it lacks the myeloid compartment.

necrosis factor (TNF)-α, and interleukin (IL)-6. Consequently, three GBM lines treated with CD11b+-conditioned media consistently showed differential expression of 16 IFN-stimulated genes, while stemness-related genes such as SOX2 or OLIG2 remained unchanged. Ultimately, the protective mechanism was tested in HDB by addition of IFN-β in permissive lines as well as in slice cultures. In both cases, ZIKV copies were robustly decreased, and the effect could be rescued using ruxolitinib, a JAK1/2 inhibitor that dampens the signaling activated in response to IFN-β.

The impact of the science is 2-fold. First, it presents a targetable mechanism that could enhance infection of glioma cells with virus particles that can be weaponized with antitumoral agents. Second, it provides a potential protective model of normal neural progenitor populations that will decrease their susceptibility against infection of viruses such as ZIKV. Further studies will be needed prior to any clinical application of these findings. In GBMs, it will need to be determined whether promoters of the infective process—such as JAK1/2 inhibitors—will be supportive of potentially oncolytic ZIKV infection without significant or dramatic side effects. From the standpoint of using the information to prevent or treat intrauterine ZIKV infection, even more work would need to be done to establish how anti-infective strategies might be employed.

Another important aspect of this paper is the scientific process used. The discovery of stem-like cells in cancer has helped bring together the worlds of cancer biology and developmental biology. Investigators in one field are using their tools and systems to apply to the other. Nowhere is this more apparent than in the study of GBM, where there are great similarities between stem-like cells in the tumors and progenitor cells in the developing brain. In their manuscript, Bulstrode et al. readily shift between studies of glioma and neural progenitors to make important observations about both brain tumors and primary ZIKV infections. We anticipate further exciting discoveries as more investigators cross these interdisciplinary boundaries.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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