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A polarizing view on posttraumatic brain injury inflammatory response

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Abstract:

Traumatic brain injury (TBI) activates the simultaneous proliferation of various pro- and anti-inflammatory molecules. Considering the amount of factors participating, this response is naturally complex. However, there is an increasing trend in neurotrauma research to delineate the injury-induced inflammatory responses within the constraints of *in vitro* defined macrophage polarization phenotypes “M1” and “M2”. Here, we evaluate research examining the complexity of the inflammatory response that cannot be so easily characterized using this binary nomenclature. TBI is demonstrated to induce a broad spectrum of simultaneous expression responses involving both pro- and anti-inflammatory reactions. Specifically, the research revealed a very heterogeneous parenchymal landscape associated with TBI. The concurrent expression of both “M1” and “M2” phenotypic markers on the microglia/macrophages involved suggests that the polarization phenotypes cannot be neatly defined in this M1/M2 paradigm. Recent studies displaying neurotrauma also report similar conflict with the constraints of this binary categorization of “M1/M2”, demonstrating that microglia/macrophages cannot effectively cross-over to strictly polarized “M1-only” or “M2-only” phenotype. Therefore, the complex signaling events surrounding this response indicate that a binary M1/M2 characterization is not adequate to define inflammatory profile. This paper is a review article. Referred literature in this paper has been listed in the references part. The datasets supporting the conclusions of this article are available online by searching the PubMed. Some original points in this article come from the laboratory practice in our research centers and the authors’ experiences.

Key words:

Categorization, inflammatory response, M1/M2, phenotype expression, traumatic brain injury

Traumatic Brain Injury Activates a Complex, Broad-Spectrum Inflammatory Response

Following traumatic brain injury (TBI), neuroinflammation is an axiomatic physiological response. Various cell types proliferate this response via the upregulation and release of soluble cellular components to the proximate tissues.^[1,2] CNS-resident microglia and astrocytes primarily produce these constituents, prolonging the activation of the innate immune response within the brain.^[3] Considering the amount of factors and receptors participating, this response is naturally complex. Despite this, many have dedicated their time and efforts to the categorization of the inflammatory response into a “M1” versus “M2” delineation as innate polarization phenotypes. Originally, studies had described innate immune polarization by examining the particular effects of singular stimuli (lipopolysaccharide,

interleukin-4, interferon), on the gene expression of the macrophages *in vitro*.^[4] The “M1/M2” terminology later evolved^[5] and expanded into subdivisions^[6-9] to appropriately adapt to the constant-changing range of stimuli and gene responses of macrophages *in vitro* over time. Overall, this effort focused on grouping tissue macrophage responses to corresponding responses of polarized lymphocytes.^[5] However, it has been revealed that the polarization states of lymphocytes^[10] do not adequately transfer to macrophages, referencing their recognizable plasticity.^[11-14] Martinez and Gordon reiterated these conclusions in a recent review^[15] proposing that, *in vivo*, the inflammatory response associated with disease or injury involves cells reacting to various stimuli concurrently, suggesting these responses involve mixed phenotypes.

Accordingly, we classified the inflammatory response of the brain within the parameters of simultaneous or mixed macrophage phenotypes after TBI. The rodent model displaying moderate

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TBI^[16] was used to characterize the temporal inflammatory profiles following trauma at various succeeding time points. The inflammatory response was examined over ninety subjects covering a wide range of the M1/M2 macrophage inflammatory spectrum, guided by exceptional sources majoring in macrophage polarization.^[6,8,11,12,15,17-21] A recent study investigated whether TBI activated a broad-spectrum inflammatory response, involving the expression of both M1 and M2 phenotypes associated with TBI.^[22] This simultaneous expression was displayed at many time points following injury, suggesting a common point of differential gene expression. In addition, microglia/macrophages in the area of trauma reflect these responses antigenically by the simultaneous expression of both M1 and M2 phenotypes on the same cell. This high level of complexity and plasticity of parenchymal macrophage responses in TBI questions the efficacy of the dichotomous system, “M1 versus M2,” that poses constraints in defining such responses.

Simultaneous M1/M2 Profiles Induced by Traumatic Brain Injury

In the study by Morganti *et al.*, they showed that TBI elicited substantial morphological changes in the innate effectors of the surrounding tissue at each time point following injury.^[22] Moreover, as a progression of time after trauma, the cells principally responsible for the production of the inflammatory mediators, macrophages/microglia, expressed a mixed phenotype by co-labeling with both polarization markers across three time points; one day, two days, and seven days following injury. The authors next demonstrated that at each time point, TBI initiated significant changes in expression of each analyte for both pro- and anti-inflammatory gene markers. Taken together, these data support the recent reports proposing that macrophages/microglia cannot effectively cross-over to a strictly polarized “M1-only” or “M2-only” phenotype. Rather, these cells display a mixed phenotype as a result of the complex signaling events that occur after injury. As such, these data show that while the macrophage/microglia population profile displays an “activated” appearance, these cells are responding to both pro- and anti-inflammatory milieu concurrently. Moreover, these techniques revealed a very heterogeneous parenchymal landscape associated with TBI, with cells displaying dually labeled “M1/M2” markers alongside “M1” and “M2” cells.

Binary characterization of microglia/macrophages is not sufficient to define inflammatory profile

Despite the complex inflammatory response discovered by microarray^[23-25] and bioinformatics,^[16,26] there is an increasing trend to adopt a binary approach to categorize these reactions based upon decade-old understanding of *in vitro*-derived stimulus responses of isolated macrophages.^[5] However, the findings by Morganti *et al.* have demonstrated that the polarization phenotypes cannot be neatly delineated in this M1/M2 paradigm, as there is a simultaneous differential expression of both “M1” and “M2” phenotypes in both the microenvironment and within the same cell.^[22] Moreover, other models displaying neurotrauma have reported similar conflict with the constraints of the binary categorization of “M1/M2”,^[27-31] even when examining a variety of differentiating factors including time course, species, and

injury location (e.g., brain or spinal cord). While the study by Morganti *et al.* admittedly does not examine every mediator previously reported to represent M1/M2 bias, the subjects studied encompass an everchanging collection of molecular mechanisms, those of which that are frequently used as animal models of neurotrauma. Moreover, as demonstrated in our data, there exists no preferential bias toward or against one polarization phenotype versus the next. This gene expression calls into question the viability of using a single antigenic marker of cell morphology (e.g., Iba1 or F4/80) to determine the inflammatory profile of these cells in a specific population. Therefore, these data show that while the macrophage/microglia population profile displays an “activated” appearance, these cells are responding to both pro- and anti-inflammatory milieu concurrently.

Conclusion

The recent findings by Morganti *et al.* align with recent works acknowledging a gap between the *in vitro* macrophage phenotype modeling and the *in vivo* tissue trauma response.^[22] Surely, these findings are by no means meant to discredit previous studies exploring M1/M2 bias after neurotrauma, recognizing the role of neuroinflammation in the propagation of neuropathophysiology following neurotrauma. Nonetheless, attempting to easily delineate the highly complex molecular mechanisms of an inflammatory response into a dichotomous nomenclature poses too many restrictions to be viable. The simultaneous differential expression of inflammatory status in this current study shows the “polarization” dogma is not applicable to TBI. While we recognize that performing large profiling experiments is not practical for every study, classification of cells (e.g. M1, M2a, M2b, M2c, M2d) depending on few selectively chosen inflammatory markers is not reasonable either in this sense. Rather, defining the roles of these markers by a neuroinflammatory sequela appears as a more pragmatic approach in characterizing the TBI-induced inflammation.

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Conflicts of interest

There are no conflicts of interest.

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