



Backhanded complement: circulating exosomes in aged animals add insult to injury after stroke

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It has long been recognized that many diseases are due to the two deadly “C’s” of either the clotting or complement systems (1) and stroke is a prime example. Stroke remains one of the leading causes of disability and death worldwide (2) and is predominantly due to thromboembolic occlusion of a major artery feeding the brain. While the initial management of stroke has understandably focused on clotting as manifest in the 1996 FDA approval of tissue plasminogen activator (tPA), often known as a “clot-busting” agent, there has been increasing recognition that other factors including inflammation are important determinants of outcome. In the new issue of *Circulation Research* (3), we learn that the complement system activated through exosomal signaling may also play a critical role after ischemic stroke. Age-associated increases in circulating inflammatory mediators have been suggested as contributors to the development of stroke and other neurological disorders (4). This notion has been supported by data showing that exposing young mice to plasma from aged mice led to cognitive functional decline (5). In contrast, heterochronic parabiosis experiments, in which the circulations of mice of different ages are connected, demonstrate that neurogenesis in aged mice can be restored by exposure to youthful circulation (6). However, the mechanism by which age-associated inflammatory mediators influence stroke remains elusive.

Zhang *et al.* (3) in *Circulation Research* investigated the role of exosomes in the pathogenesis of stroke. Compared to young controls (3-month-old), exosomes isolated from

aged rats (21- to 23-month-old) serum had larger diameters and lower concentrations. Aged rats were subjected to a simulated stroke with permanent distal middle cerebral artery occlusion (dMCAO). With the blood-brain-barrier (BBB) disrupted, intravenous injection of exosomes from aged rats were found in healthy and ischemic brain regions (the striatum, hippocampus, and cerebellum) for 14 days. Using cell lineage markers, the authors found that these injected exosomes had accumulated within neurons, microglia, and endothelial cells. These results indicate that after a stroke, exosomes from aged subjects cross a disrupted BBB and enter neuronal and non-neuronal populations within the brain.

How do exosomes from younger and older rodents influence simulated stroke outcomes during aging? To determine the role of exosomes in stroke during aging, aged rats were subjected to dMCAO. Injection of exosomes isolated from young rat serum reduced infarct volume and improved sensorimotor deficits 72 h after stroke with effects lasting for as long as 35 days. In contrast, injection of exosomes isolated from aged rat serum worsened infarction and sensorimotor deficits in aged rats. These data provide evidence that exosomes influence post-stroke outcomes in aged subjects and that exosomes from younger and older rodents have fundamentally different effects on the aged brain. Notably, young rats subjected to stroke saw no change in stroke volume or function after infusion of serum exosomes from old rats.

The investigators found that the beneficial and harmful

effects of exosomes may have a cellular basis centered not on the neuron, but the microglia which are known to play key roles in the primary immune responses within the central nervous system. First, they found that activated microglia resided in the penumbra and that aged exosomes increased the activated population. Second, they found that the activated microglial population took on a classical M1 proinflammatory activation pattern (*Iba⁺CD86⁺*) and not a protective M2 phenotype (*Iba⁺CD206⁺*). Third, aged exosome exposure led to worsened postinfarction synaptic activity with a reduction in the total length and number of dendritic spines in area neurons. Interestingly, exosomes (from young or old plasma) did not influence resting neuronal potentials. However, exosomes from young animals were able to reverse microglial activation. Finally, a series of elegant experiments reveal that microglial depletion using a small molecule inhibitor of CSF1R was able to reduce brain injury and improve motor performance in older postinfarction rodents. Taken together, the authors conclude that aged exosomes serve to activate the microglial population which subsequently injures the neuronal population through synaptic phagoptosis while young exosomes appear to beneficially reverse microglial activation. The authors are to be congratulated on rigorously examining, for the first time, the role of exosomes from young and aged subjects in the pathogenesis of stroke. This work highlights new areas of study concerning the therapeutic potential of circulating exosomes in elderly patients after stroke.

How do exosomes from young and older rodent populations differ? To further delineate the molecular mechanisms governing exosomal exacerbation of stroke, Zhang *et al.* (3) compared protein profiles in exosomes from young and aged rats. One hundred and twenty-six proteins were differentially expressed (71 upregulated and 55 downregulated) and were enriched for gene ontology categories that broadly involved inflammation, phagoptosis, and complement activation. Among 126 differently expressed proteins, three complement proteins (CD46, C3a, and C3b) were further validated by western blot, where CD46 has higher and C3a and C3b had lower expression levels in exosomes from young rats compared to aged rats. In aged rats subjected to dMCAO, compared to injection of exosomes from young rats, these complement proteins, C3a/C3b and C3a receptor (C3aR), were increased in active microglia in the penumbra 48 and 72 h after injection of exosomes from aged rats. These data suggest an injection of exosomes from aged rats may exacerbate poststroke brain

injury by inducing C3a/C3b and C3aR in active microglia. Indeed, in aged rats subjected to dMCAO and injection of exosomes from aged rats, application of C3aR inhibitor, SB290157, reduced activated microglia in the penumbra and decreased phagoptosis as well as reducing poststroke synaptic damage and infarction, leading to improvement of sensorimotor performance. Taken together, these data suggest an essential role of C3aR, and more generally complement proteins, in the development of stroke in aged subjects. However, it is still unclear whether these beneficial effects of C3aR inhibitor are through inhibiting C3aR locally in the brain or C3aR carried by the injected exosomes.

This interesting work (3) provides new insights into the contribution of exosomes in the development of stroke in aged subjects. First, the BBB is disrupted during stroke in aged subjects, allowing exosomes to cross and deliver proinflammatory mediators that exacerbate stroke outcomes. Second, through the delivery of complement system proteins (e.g., C3a/C3b, C3aR) blood exosomes regulate microglial phagoptosis, stroke-induced brain damage, and sensorimotor performance. Third, microglial depletion by inhibition of C3aR is effective in attenuating stroke-induced brain damage in aged subjects, suggesting that C3aR could be a therapeutic target for the treatment of stroke. Finally, exposure of aged rats to young blood exosomes reversed poststroke synaptic and neurological functional declines, indicating a therapeutic anti-inflammatory potential for the application of young blood exosomes in protecting the brain against stroke.

As with all interesting and provocative work, multiple questions are raised by Zhang *et al.* (3) that warrant further investigation. First, since exosomes can be secreted by multiple organs/tissues and multiple cell types (7), identifying the source(s) of circulating exosomes and the changes seen in aging would be of great interest and might enable complementary therapeutic strategies blocking exosome release. Second, exosomes carry multiple bioactive molecules in addition to proteins examined here, including coding and noncoding RNAs as well as DNA (8). It seems likely that other exosome molecular cargoes are also contributing to the age-related effects observed. Third, although Zhang *et al.* (3) identified complement system members as contributing to the adverse effects of old exosomes, the drivers of the benefits seen with young exosomes remain largely undefined. Fourth, since stroke can alter the profile of exosome synthesis and secretion (9) and the current study focused on injection of

exogenous exosomes, more work will be needed to parse the roles of endogenous exosomes at different timepoints in this process. Finally, before clinical translation can be investigated, demonstration of similar exosome biology in human stroke as well as an understanding of how exosomes might be altered by reperfusion (still the mainstay of ischemic treatment) or patient behaviors and co-morbidities will be essential.

Despite these issues, Zhang *et al.* (3) have admirably provided evidence of the contribution of exosomes in a rat model of stroke-induced brain injury and have expanded our understanding of ischemic stroke beyond clotting to include a key role for the complement system (e.g., C3aR) in the postinfarction process. These findings provide valuable insight into the molecular correlates of stroke during aging with possible implications for therapeutic intervention among our aging population. Exploring the potential of exosomal, or C3aR specific interventions, for the treatment of stroke will be an exciting opportunity for future investigation.

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