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COMMENTARY

Insights into End-Organ Injury in HIV Infection

Dynamics of Monocyte Trafficking to the Brain in SIV Encephalitis

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Neurocognitive impairment (collectively named as HIV-1-associated neurocognitive disorders or HAND) is highly prevalent in HIV-1-infected patients, including those receiving antiretroviral therapy (ART).¹ Monocyte and macrophage infiltration in the brain is associated with HAND development²; however, precise timing and dynamics of this infiltration remain debatable. Researchers recapitulate HIV infection in nonhuman primates (macaque model) by infecting with SIV. For the first time, by using SIV-infected animals that developed SIV encephalitis (SIVE), Nowlin et al³ studied monocyte-to-macrophage turnover in the central nervous system (CNS) under physiological conditions.⁴

Study Design

Neuropathologic features of SIVE recapitulate signs of HIV encephalitis,⁴ a morphological correlate of cognitive decline. Nowlin et al³ used straightforward multilabel immunohistochemical approaches to assess macrophage populations and their turnover in the choroid plexus, meninges, perivascular space, and parenchymal lesions (when present in animals with SIVE). The method also allowed comparison of the rates of turnover in early acute inflammation and in the later inflammation as the animals developed AIDS and CNS lesions. They used a cutting-edge combination of bromodeoxyuridine (BrdU)—labeled macrophage precursors in bone marrow (that can potentially traffic to the brain) to detect macrophage populations and the serial injection of dextran dyes intercosternally to determine the macrophage populations (early or late)

contributing to lesion formation as well as to identify the infected cells in the CNS.

Study Outcome

The findings of Nowlin et al³ are of great interest to CNS macrophage biologists as well as immunologists studying acute and chronic inflammation. Early during SIV infection, Nowlin et al³ reported most cells to be MAC387⁺ recently recruited monocytes/macrophages. As expected, most of these cells were detected in the meninges and the choroid plexus (which appeared to have trafficking kinetics similar to the blood than to the CNS compartments). Consistent with the observations from the bone marrow during inflammation, most BrdU⁺ cells were also MAC387⁺.

During the late stages of inflammation, Nowlin et al³ found macrophage accumulation primarily in the perivascular space and in SIVE lesions, not in the meninges and choroid plexus. It is likely that the meninges and choroid plexus have cells trafficking through them in late stages of inflammation, thereby decreasing accumulation. Interestingly, most cells in the SIVE lesion (>80%) were present in the CNS before lesion development, suggesting

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migration of the cells to the lesions from other CNS vessels and perivascular sites, as opposed to, or with, cells trafficking to the CNS. These data are consistent with their observations that fewer, if any, BrdU⁺ perivascular macrophages were observed. It is likely that these cells turn over in the CNS, as has been suggested by studies in rodents.⁵ By using dextran dye scheme, the authors³ demonstrated a 2.9-fold increase in the percentage of infected macrophages that later enter the CNS.

These newly described intriguing observations have not been reported in either a lentiviral infection model or a nonhuman primate model, underlining the fact that CNS inflammation is a dynamic process. Nowlin et al³ reported an increased ratio of CD163⁺ (with phenotype of M2 alternatively activated macrophages⁶)/MAC387⁺ macrophages (that might have an M1 phenotype) in the CNS of animals with SIVE.

In HIV-infected humans and SIV-infected monkeys, the index of BrdU⁺ monocytes in blood (some of which are CD163⁺ cells) and/or soluble CD163 (sCD163) are markers for the following: i) the speed of developing AIDS and the severity of tissue pathogenesis (macrophage inflammation, tissue damage, and number of multinucleated giant cells)⁷; ii) the number of inflammatory macrophages in the CNS of infected monkeys; iii) the presence of noncalcified vulnerable cardiac plaques in HIV-infected individuals receiving durable ART and elite controllers⁸; iv) the level of macrophage accumulation in the ascending aorta of HIV-infected humans and SIV-infected monkeys⁹; and v) the level of neurocognitive deficits in HIV-infected patients receiving durable ART.⁷

Consistently, Nowlin et al³ demonstrate that the rate of recruitment of the CD163⁺ macrophages to the CNS is increased with SIV infection, correlating with the rapid death. In addition, the increased monocyte activation (measured by sCD163 and/or the average CD14⁺CD16⁺ monocyte count) is associated with greater CD163⁺ macrophage recruitment and SIVE. In HIV-infected patients, such cells may be key players in the conditions collectively known as serious non-AIDS events that now represent the major cause of mortality in HIV-infected individuals with virus suppression.¹⁰

So, in addition to the finding that most CD163⁺ macrophages in SIVE lesions arise from possible redistribution of perivascular cells present in the CNS before the development of lesions, clearly monocyte/macrophage activation and turnover from bone marrow and transit in the blood (where sCD163 is shed with activation) are also predictors of pathogenesis in acute and chronic inflammation. This chronic inflammation and cell trafficking likely contribute cyclically to chronic immune activation. By using an anti—very late after activation antigen 4 antibody (natalizumab), it has been recently demonstrated¹¹ that blocking monocyte traffic to the CNS and leukocyte traffic to the gut early stops seeding of the CNS with virus and, interestingly, results

in nondetectable bacterial translocation (low levels of lipopolysaccharide in plasma). Similar antibody treatment during late SIV infection, with ongoing CNS inflammation, lesions, and neuronal injury, diminishes neuronal injury and decreases or eliminates SIVE lesions.

Altogether, these data suggest that monocyte/macrophage traffic and accumulation in the CNS are required for continued lesion formation, and peripheral markers of myeloid cell activation (including sCD163 made only by myeloid cells) serve as biomarkers of this process. Recent research¹² suggests that the cells trafficking into the CNS can potentially traffic out, complicating assessment with regard to monocyte accumulation and turnover. Although the investigators mainly focus on monocyte subtype patterns, accumulation and retention could be dependent on changes in the cells composing the blood-brain barrier (brain endothelium, pericytes, and astrocytes), which contribute to monocyte and lymphocyte migration in the CNS.¹³

Future Directions

Whether MAC387⁺ cells correspond to a proinflammatory M1 phenotype and CD163⁺ macrophages are the alternatively activated M2 phenotype require further investigation because overlapping features with the two entities are suggested. In humans and monkeys, there are insufficient markers to distinguish between the intermediate activation states.⁶ As emphasized earlier, such cells demonstrate numerous phenotypes and participate in inflammation initiation, its resolution, phagocytosis, pathogen killing, angiogenesis, and tissue regeneration. Both M1 and M2 cells may possess either beneficial or pathological consequences, depending on the pathological process.

The findings of Nowlin et al³ are vital for a better understanding of HAND pathogenesis because its prevalence remains high (17% to 50%)¹⁴ in patients receiving ART. The underlying causes of HAND (the complication most feared by infected individuals) are believed to be associated with chronic neuroinflammatory responses,¹⁵ the generation of proinflammatory factors by activated macrophages and microglia, and persistent injury of the blood-brain barrier.¹⁶

Virus appears in the CNS weeks after HIV infection and coincides with the appearance of inflammatory molecules in the cerebrospinal fluid.¹⁷ Virus then resides in the perivascular monocytes/macrophages that are infected in the periphery.⁴ Before introduction of ART, an uncontrolled infection in the brain leads to HIV-1 encephalitis, which occurs in immunosuppressed patients because of the depletion of CD4⁺ T lymphocytes, thereby resulting in inadequate immune control over HIV-1 replication in the CNS and other organs.¹⁸ Diagnostic hallmarks of HIV-1

encephalitis include brain atrophy, perivascular infiltrates composed of mononuclear phagocytes, microglial nodules, reactive astrocytosis, the presence of multinucleated giant cells, and myelin pallor.²

HIV-1 encephalitis stimulates neurodegeneration by triggering glutamate excitotoxicity, axonal damage, and neuronal apoptosis through the production of proinflammatory factors and viral proteins released from infected cells.² Moreover, HIV-1 induces activation of astrocytes and microglia, which produce soluble neurotoxic factors within the CNS.¹⁶ Prolonged activation of perivascular macrophages and microglia in the CNS contributes to HIV-1 neuroinflammation and is associated with neurodegeneration and neurocognitive impairment.¹⁶ Although host-specific differences exist between HIV and SIV infection, the macaque model provides a more relevant model for human disease as compared to other models (like humanized mouse models where infectious HIV-1 is used),¹⁹ which do not allow studies of macrophage turnover in the natural host, limiting their utility for studies performed by Nowlin et al.³

Findings by Nowlin et al.³ also emphasize that development of SIVE is a late event in the infection process, secondary to the loss of immune function and lack of virus suppression in the periphery.¹⁸ Monocytes (particularly more mature CD16⁺ monocytes)²⁰ can be infected with HIV. Their pattern of migration or organ infiltration might be different from other subpopulations in peripheral blood. More important, such cells can be the key players in end-organ injury during HIV and SIV infection. When differentiated into long-living tissue macrophages, these cells become a virus reservoir that is difficult (if not impossible) to eradicate.¹⁶ This is especially true for CNS macrophages protected by the blood-brain barrier that is poorly penetrable by antiretroviral drugs.

Conclusions

It is now evident that sCD163 is useful as a biomarker of macrophage activation in various inflammatory diseases, such as macrophage activation syndrome, sepsis, and liver disease. sCD163 is a general risk marker of comorbidity and mortality in several chronic inflammatory disease states.²¹ sCD163 may serve as both an indicator of disease activity and a biomarker of treatment success. The study makes an important point regarding monocyte activation and trafficking in the CNS as potential targets for therapeutic intervention. Anti-inflammatory interventions dampening monocyte activation and diminishing their migratory potential could be neuroprotective and prevent other organ injury in HIV infection.¹⁶

In summary, the work of Nowlin et al.³ provides key new data on the development of SIVE, potentially guiding new

treatment approaches for HAND—namely, prevention of monocyte CNS infiltration in HIV-1 infection.

References

- McArthur JC, Steiner J, Sacktor N, Nath A: Human immunodeficiency virus-associated neurocognitive disorders: mind the gap. *Ann Neurol* 2010, 67:699–714
- Cherner M, Masliah E, Ellis RJ, Marcotte TD, Moore DJ, Grant I, Heaton RK: Neurocognitive dysfunction predicts postmortem findings of HIV encephalitis. *Neurology* 2002, 59:1563–1567
- Nowlin BT, Burdo TH, Midkiff C, Salemi M, Alvarez X, Williams KC: SIV encephalitis lesions are composed of CD163⁺ macrophages present in the central nervous system during early SIV infection and SIV-positive macrophages recruited terminally with AIDS. *Am J Pathol* 2015, 185:1649–1665
- Williams KC, Corey S, Westmoreland SV, Pauley D, Knight H, deBakker C, Alvarez X, Lackner AA: Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuro-pathogenesis of AIDS. *J Exp Med* 2001, 193:905–915
- Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M: Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 2010, 330:841–845
- Shechter R, Schwartz M: CNS sterile injury: just another wound healing? *Trends Mol Med* 2013, 19:135–143
- Burdo TH, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, Rosenberg ES, Ellis RJ, Williams KC: Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. *J Infect Dis* 2011, 204:154–163
- Tawakol A, Grinspoon SK: Imaging atherosclerotic burden and inflammation: insights into the spectrum of atherosclerotic disease in HIV. *J Nucl Cardiol* 2015, 22:381–384
- Subramanian S, Tawakol A, Burdo TH, Abbara S, Wei J, Vijayakumar J, Corsini E, Abdelbaky A, Zanni MV, Hoffmann U, Williams KC, Lo J, Grinspoon SK: Arterial inflammation in patients with HIV. *JAMA* 2012, 308:379–386
- Hsu DC, Sereti I, Ananworanich J: Serious non-AIDS events: immunopathogenesis and interventional strategies. *AIDS Res Ther* 2013, 10:29
- Campbell JH, Ratai EM, Autissier P, Nolan DJ, Tse S, Miller AD, Gonzalez RG, Salemi M, Burdo TH, Williams KC: Anti- α 4 antibody treatment blocks virus traffic to the brain and gut early, and stabilizes CNS injury late in infection. *PLoS Pathog* 2014, 10:e1004533
- Alvarez X, Midkiff C, Lackner AA, Williams KC: Do SIV infected macrophages leave the CNS? 32nd Annual Symposium on Non-Human Primate Models for AIDS, Portland, OR, November 11–14, 2014
- Hill J, Rom S, Ramirez SH, Persidsky Y: Emerging roles of pericytes in the regulation of the neurovascular unit in health and disease. *J Neuroimmune Pharmacol* 2014, 9:591–605
- Su T, Schouten J, Geurtsen GJ, Wit FW, Stolte IG, Prins M, Portegies P, Caan MW, Reiss P, Majoie CB, Schmand BA; AGEHIV Cohort Study Group: Multivariate normative comparison, a novel method for more reliably detecting cognitive impairment in HIV infection. *AIDS* 2015, 29:547–557
- Kamat A, Lyons JL, Misra V, Uno H, Morgello S, Singer EJ, Gabuzda D: Monocyte activation markers in cerebrospinal fluid associated with impaired neurocognitive testing in advanced HIV infection. *J Acquir Immune Defic Syndr* 2012, 60:234–243
- Persidsky Y, Ho W, Ramirez SH, Potula R, Aboud ME, Unterwald E, Tuma R: HIV-1 infection and alcohol abuse: neurocognitive impairment, mechanisms of neurodegeneration and therapeutic interventions. *Brain Behav Immun* 2011, 25(Suppl 1):S61–S70

17. Suh J, Sinclair E, Peterson J, Lee E, Kyriakides TC, Li FY, Hagberg L, Fuchs D, Price RW, Gisslen M, Spudich S: Progressive increase in central nervous system immune activation in untreated primary HIV-1 infection. *J Neuroinflammation* 2014, 11:199
18. Potula R, Poluektova L, Knipe B, Chrastil J, Heilman D, Dou H, Takikawa O, Munn DH, Gendelman HE, Persidsky Y: Inhibition of indoleamine 2,3-dioxygenase (IDO) enhances elimination of virus-infected macrophages in an animal model of HIV-1 encephalitis. *Blood* 2005, 106:2382–2390
19. Gorantla S, Poluektova L, Gendelman HE: Rodent models for HIV-associated neurocognitive disorders. *Trends Neurosci* 2012, 35:197–208
20. Jaworowski A, Kamwendo DD, Ellery P, Sonza S, Mwapasa V, Tadesse E, Molyneux ME, Rogerson SJ, Meshnick SR, Crowe SM: CD16+ monocyte subset preferentially harbors HIV-1 and is expanded in pregnant Malawian women with *Plasmodium falciparum* malaria and HIV-1 infection. *J Infect Dis* 2007, 196:38–42
21. Moller HJ: Soluble CD163. *Scand J Clin Lab Invest* 2012, 72:1–13