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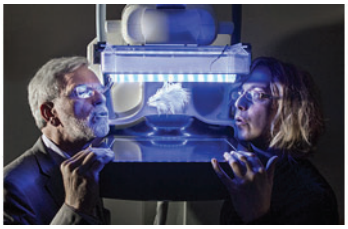
Collaboration Opportunity

Novel insights into neuroinflammatory diseases from studying freshly isolated human microglia and CNS-surveilling T cells

CNS, neuroinflammation, brainbank, T cells, microglia, expression profiling, functional assays 2015

Background

Innate and adaptive immune cells are crucially involved in the onset and progression of neuroinfl ammatory diseases as multiple sclerosis (MS), yet their study is hampered by limited access of primary human cells for research.

We recently developed a method for rapid isolation of pure microglia and T cells from post-mortem human brain tissue that omits effects of prolonged culture (and adherence) and therefore allows for observations on phenotypic and functional features that likely refl ect the in vivo biology of these cells in a highly accurate way. Brain tissue is obtained in the framework of the Netherlands Brain Bank (NBB) that performs 130 brain autopsies annually with a very short post-mortem delay of 4-8 hours. We found that microglia from normalappearing white matter (NAWM) display CD45 expression levels and scatter characteristics that are signifi cantly lower compared to those of autologous peripheral macrophages from the choroid plexus. The phenotype of isolated microglia was further specified by absent surface expression of CD14, CD200 receptor, and mannose receptor (CD206), all of which were markedly expressed by macrophages. Importantly, we showed that absent CD14 protein expression coincides with a lack of LPS responsiveness in primary human microglia, indicative of a profoundly immunosuppressed state, whereas clear responses to IL-4 and dexamethasone were found.

Microglia isolated from NAWM of MS expressed increased amounts of CD45 and CD32b and had increased size and granularity as compared to controls without neurological disease, but could clearly be distinguished from peripheral macrophages by lack of CD206. In spite of the activated phenotype in MS, they were completely unresponsive to LPS, whereas they did respond, though different, to antiinfl ammatory stimuli such as dexamethasone and IL-4.

These data indicate that microglia in NAWM of MS patients are in a state of alertness that comprises changes in morphology and gene expression but also persistence of efficient immunosuppressive mechanisms.

The immediate post-mortem isolation approach of microglia also enabled us to isolate and phenotype primary human T cells from brain tissue. We showed that CNS associated T cells make up a population of tissue-adapted long-lived effectortype T cells, most of them being CD8+, which use CX3CR1 to home in the perivascular space. Notably, protein expression of the cytolytic enzymes perforin, granzyme A, and granzyme B was relatively low, indicating that the cells reside in a tightly regulated suppressive environment, possibly enabling a fast response to local threats.

The Technology

Brain tissue will be collected from donors of the Netherlands Brain Bank, a department of the Netherlands Institute of Neuroscience (www.brainbank.nl). Annually, 30 control donors, 10-15 MS donors, 2-5 donors without neurological disease and with sepsis, and 2-8 donors with neurological disease and sepsis come to autopsy, all with extreme low post mortem delays (4-8 hours). All clinical records of brain donors are analyzed, and extensive anonymized clinical summaries are provided with the tissue, indicating all clinical characteristics, intoxications, and complete records of drug use. This will allow to dissect effects of anti-inflammatory treatment and peripheral inflammation from central infl ammation. Extensive neuropathological diagnoses will complete the information on the donors. Coronal slices of MS brains are scanned post mortem by MRI to guide tissue dissection of NAWM or lesions in MS.

Cells will be obtained from different brain regions such as normal appearing white matter (corpus callosum, subcortical white matter) and normal appearing grey (cortical) matter, but also from chronic active and inactive MS lesions. Pure cell populations are obtained by a combination of tissue dissociation and density gradient separation and are extensively phenotyped directly using multi-color flowcytometric analysis for cell surface marker expression and cytokine production. Using magnetic bead separation, we will isolate highly enriched and viable populations of ≥500.000 microglia or T cells per donor,

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with purities of >97% and often more than 99%, for genome wide gene expression profiling and functional assays immediately upon isolation.

To provide a general measure of immune activation in the local brain tissue, CD45 expression and scatter characteristics of the isolated microglia will be determined, and CD45 mRNA expression will be quantifi ed in callosal parenchyma of all donors. To define specific features of MS or other diseases, donors with peripheral infl ammatory conditions (eg, sepsis, pneumonia, severe wound infection, end-stage chronic obstructive pulmonary disease, and infections) will be dissected from the control and MS groups.

Applications

The worldwide unique setting in Amsterdam of the Netherlands Brain Bank with many MS and control autopsies with very low post mortem delay and research groups specialized in microglia and T cell biology will facilitate research directed at unraveling pathogenic mechanisms in MS tissue and identifying pathways relevant for the development of effective therapeutic approaches to treat MS. Phenotyping and profiling pure primary microglia and T cells in MS will provide for the first time a wealth of information on mechanisms underlying MS lesion formation; it will identify early microglia alterations and macrophage phenotypes in relation to demyelination. Also, clonality, cytokine profile, and antigen specificity of T cells infiltrating the MS brain finally will come in reach now, possibly revealing the actual cause of MS.

Potential applications are:
1. *FACS analysis* for profiling of cell surface marker expression and cytokine production of microglia and T cells. Parallel analysis of ≥11 markers will guarantee optimal usage of the limited material
2. *Proteome analysis* using the SYNAPT™ G2 platform (www.waters.com) that combines quantitative Tof (time of flight) technology with enhanced High Definition MS (mass spectometry) technology to provide highest performance
3. *Transcriptome analysis* of mRNAs and microRNAs by microarray (www.servicexs.com) of the isolated cells to establish a full gene expression profile and pathway analysis. mRNA for microarray analysis is currently collected from microglia of all non-septic MS and control donors that come to autopsy
4. *Epigenetic profiling* by chromatin immunoprecipiation (ChIP) of nuclear extracts from the isolated cells and by FACS assays
5. *Functional assays* focusing on phagocytosis and inflammatory responses of microglia. Uptake of fluorescently labeled myeline will be analyzed to establish phagocytosis capacity. This will be combined with assessment of the inflammatory potential of microglia by activation (e.g. with LPS, IL-4, IL-10 or glucorticoids) and subsequent analyses of cytokine production, either pro-infl ammatory or antiinflammatory and surface marker expression
6. *Further applications* include biomarker testing, infection models, signaling studies, and pharmacological assyas
7. *Validation* Since its start in 1985, the NBB has performed almost 3500 autopsies, of which 200 MS. Therefore, many frozen and paraffin blocks of MS lesions and NAWM are available for research. Most of these lesions have been extensively characterized and provide all possibilities for target validation.

Ethical and legal use of donor tissue and medical records for research purposes is ensured (www.brainbank.nl).

Inventors

Our research is jointly performed by the Department of Experimental Immunology (Dr. Jörg Hamann and Prof. René van Lier) at the Academic Medical Center of the University of Amsterdam (www.amc.nl) and the Neuroimmunology Group (Dr. Inge Huitinga) at the Netherlands Institute for Neuroscience (www.nin.knaw.nl). Dr. Inge Huitinga is also the director of the Netherlands Brain Bank.

Key Publications

Melief J, Koning N, Schuurman KG, Van De Garde MD, Smolders J, Hoek RM, van Eijk M, Hamann J, Huitinga I. Phenotyping primary human microglia: tight regulation of LPS responsiveness. Glia. 2012 Oct;60(10):1506-17. PubMed PMID: 22740309.

Melief J, Schuurman KG, Van de Garde MD, Smolders J, van Eijk M, Hamann J, Huitinga I. Microglia in normal appearing white matter of multiple sclerosis are alerted but immunosuppressed. Submitted for publication.

Smolders J, Remmerswaal EBM, Schuurman KG, Melief J, van Eden CG, van Lier RAW, Hamann J, Huitinga I. T cell surveillance of the human central nervous system. Submitted for publication.

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