Molecular Neurodegeneration

PERSPECTIVE

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$CD8^+$ T cells in neurodegeneration: friend or foe?

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Main text

T cell infiltration is enhanced in disease-associated brain areas in neurodegenerative diseases such as multiple sclerosis (MS), Alzheimer's disease (AD) and Parkinson's disease (PD), with most of them being $CD8^+$ T cells [1, 2]. Increased clonal expansion and heightened expression of T cell activation and cytotoxicity-associated genes in clonally expanded $CD8^+$ T cells in the cerebrospinal fluid (CSF) are reported in patients with MS, AD or PD. [3–5] Clonal expansion of T cells indicates T cell recognition of a specific antigen and subsequent establishment of an immune response. $CD8^+$ T cells are commonly viewed as pro-inflammatory cytotoxic T lymphocytes (CTLs) even though immune-suppressive $CD8^+$ regulatory T cells ($CD8^+$ Tregs) have been described for over a half centenary [6]. Therefore, it is understandable to conjecture that these disease-associated $CD8^+$ T cells elicit immune responses and inflict cytotoxicity in the central nervous system (CNS) resulting in neurodegeneration [3, 4]. However, recent single-cell RNA sequencing (scRNA-seq) and single-cell T-cell receptor sequencing (scTCR-seq) analyses in conjunction with flow cytometric analysis reveal that both clonally expanded $CD8^+$ T cells in neurodegenerative diseases [4, 5, 7] and immune suppressive $CD8^+$ Tregs [8, 9] are terminally differentiated effector memory T cells (T_{EMRA}) expressing high levels of cytotoxicity-associated molecules and sharing cell surface markers, raising the critical question of what role these clonally expanded $CD8^+$ T cells play in neurodegenerative diseases. Here we discuss the phenotype and function of clonally expanded $CD8^+$ T cells in neurodegenerative diseases and immune suppressive $CD8^+$ Tregs and postulate their roles in neurodegeneration.

TCR Vβ repertoire analysis in MS patients shows that clonally expanded $CD8^+$ T cells in MS lesions in the brain are reflected in peripheral blood and CSF, particularly, in CSF [10]. Therefore, analyzing clonally expanded $CD8^+$ T cells in CSF, which is much more feasible than analyzing the sparse brain infiltrating T cells, is a valuable approach to study the role of T cells in neurodegenerative diseases. The recent advance in scTCR-seq and scRNA-seq techniques enables one to simultaneously measure TCR and gene expression profiles at single-cell resolution, which not only allows identifying clonally expanded $CD8^+$ T cells in AD, PD and MS, but also reveals functional and physiological insights in these cells through analyzing corresponding global gene expression profiles [4, 5, 7]. CD45RA is a naïve T cell marker, but T_{EMRA} cells regain the expression of CD45RA while maintaining the $CD27^-CCR7^-$ cell surface marker characteristic of effector memory cells. Therefore, T_{EMRA} cells are conventionally defined as $CD45RA^+CD27^-$, $CD45RA^+CCR7^-$, or $CD45RA^+$ $CD27^-CCR7^-$ T cells, which can be readily identified with flow cytometric analysis using fluorescent-conjugated antibodies specific for CD45RA, CD27 or CCR7. scRNA-seq analysis does not usually distinguish the RA and RO isoforms of CD45, and T_{EMRA} cells are defined as memory ($CD27^-CCR7^-$) T cells expressing high levels of T_{EMRA}-associated genes such as *GZMA*

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(granzyme A), *GZMB* (granzyme B), *PRF1* (perforin) and *NKG7* [5]. T_{EMRA} cells are highly cytolytic but have a poor proliferation capacity. Flow cytometric analysis shows that circulating $CD8^+$ T cells in MS tend to acquire a terminally differentiated phenotype [10], and $CD8^+$ T_{EMRA} cells are also increased in peripheral blood and CSF in AD, and are negatively associated with cognition [4]. scRNA-seq and scTCR-seq analyses in conjunction with flow cytometric analysis of CSF cells reveal that clonally expanded $CD8^+$ T cells from AD patients are $CD45RA^+CD27^-CCR7^{-/low}CD127^-CD161^-PD\text{-}1^-$ T_{EMRA} cells expressing high levels of granzyme genes, *NKG7*, *CST7*, *CCL4* and *CCL5* [4]. scRNA-seq and scTCR-seq analyses of CSF cells isolated from patients with PD show clonally expanded T cells were enriched in $CD27^-CCR7^-GZMA^{hi}GZMB^{hi}PRF1^{hi}NKG7^{hi}$ $CD8^+$ T cells, and these cells also express high levels of *CCL5*, *CST7*, *GZMH* and *GZMK* [5]. scRNA-seq and scTCR-seq analyses show that clonally expanded CD8 T cells in MS express higher levels of CD8 effector function–related molecules including granzymes A and K, *NKG7*, *PFN1*, *CST7*, *CCL5*, and *CCL4* and express lower levels of *SELL* (CD62L), which are $CCR7^{-/low}CD127^-$ [7]. These gene expression characteristics indicate that, like in AD patients, clonally expanded $CD8^+$ T cells in the CSF of patients with PD or MS are also T_{EMRA} cells. However, gene expression analysis does not reveal the cellular function of these cells. By consensus, $CD8^+$ T_{EMRA} cells have high cytotoxicity and lyse target cells as a regular cytotoxic T lymphocyte (CTL) via T-cell receptor (TCR) recognition of a specific peptide presented by a compatible MHC molecule. Therefore, it is postulated that these clonally expanded $CD8^+$ T_{EMRA} cells are highly proinflammatory and promote neurodegeneration [4].

Interestingly, the cell surface marker expression of clonally expanded $CD8^+$ T_{EMRA} cells in the CSF of patients with AD is similar to that of $CD161^-CD56^+$ $CD8^+$ Tregs identified in human peripheral blood a decade ago [11, 12]. Both are $CD45RA^+CD27^-CCR7^{-/low}CD127^-CD161^-PD\text{-}1^-$, though it is not specified whether these clonally expand $CD8^+$ T_{EMRA} cells are $CD56^+$. $CD161^-CD56^+$ $CD8^+$ Tregs kill TCR-activated effector $CD4^+$ T cells showing functional and cell surface marker similarities to the recently identified immune-suppressive KIR^+CD8^+ T cells, the human equivalent of mouse $Ly49^+CD8^+$ Tregs that prevent or dampen autoimmune responses [8, 13, 14]. Both $CD161^-CD56^+$ $CD8^+$ Tregs and KIR^+CD8^+ T cells are $CD45RA^+CD27^-CCR7^-CD28^-CD127^-$, and kill activated $CD4^+$ T cells in a cell-cell contact-dependent manner. It has been reported that almost all KIR^+ T cells are $CD56^+$, and the majority of KIR^+ T cells are $CD8^+$ T cells [15]. These observations indicate that $CD161^-CD56^+$ $CD8^+$ Tregs and immune-suppressive KIR^+CD8^+ T cells are likely the same immune regulatory $CD8^+$ T cell subpopulation. Since $CD161^-CD56^+$ $CD8^+$ Tregs and KIR^+CD8^+ T cells are $CD45RA^+CD27^-CCR7^-$, they should be considered as $CD8^+$ T_{EMRA} cells following the conventional classification. Moreover, scRNA-seq and scTCR-seq analyses show that, like clonally expanded $CD8^+$ T cells in neurologic diseases, clonally expanded KIR^+CD8^+ T cells express elevated levels of *GZMH*, *GZMB*, and *PRF1* [8]. Increased KIR^+CD8^+ T cells are found in the peripheral blood and inflamed tissues of patients with autoimmune diseases including MS and during viral infection. However, KIR^+CD8^+ T cells do not seem to be induced to aggravate the autoimmunity. Instead, KIR^+CD8^+ T cells are shown to specifically kill activated pathogenic or autoreactive $CD4^+$ T cells acting as immune-suppressive regulatory T cells [8]. Moreover, unlike conventional T_{EMRA} cells that are terminally differentiated with poor proliferative capacity, $CD161^-CD56^+$ $CD8^+$ Tregs proliferate robustly and maintain their functional characteristics after long-term culturing [11, 12]. Clearly, $CD161^-CD56^+$ $CD8^+$ Tregs and KIR^+CD8^+ T cells do not fit into the conventional concept of $CD8^+$ T_{EMRA} cells. Thus, the key question is whether clonally expanded $CD8^+$ T_{EMRA} cells in AD, PD, or MS are immune-suppressive regulatory cells that kill pathogenic $CD4^+$ T cells, or they are proinflammatory cytolytic cells that fuel neurodegeneration.

The categorization of T_{EMRA} cells is more a developmental stage classification than a cell lineage/type definition. Unlike $CD4^+$ T cells that have well-defined transcription factors, cell surface markers and cytokines to categorize the population into subtypes such as Th1, Th2, Th17, Tfh, Tregs, etc., the sub-classification of $CD8^+$ T lineages is still vague. With currently available gene expression information, it cannot be determined whether these clonally expanded $CD8^+$ T_{EMRA} cells and $CD8^+$ Tregs are completely or partially overlapping or non-overlapping populations. Theoretically, $CD8^+$ T_{EMRA} cells in disease-impacted brain areas could be either CTLs that promote detrimental immune responses or regulatory cells that are induced by the undesired immune responses in the brain to dampen the detrimental immune responses, or a mixture of both cell types. The shared cell type-defining surface markers of terminally differentiated CTLs and immune-suppressive $CD8^+$ Tregs signify the necessity for new $CD8^+$ T cell classification markers. Studies dedicated to identifying the diversity of $CD8^+$ T_{EMRA} cells and each subpopulation's biological function are needed to fill in a blank spot in immunology. Interestingly, single-cell trajectory analysis shows that the terminal effector $CD8^+$ T cells (T_{EMRA} cells) in the CSF from PD patients display two

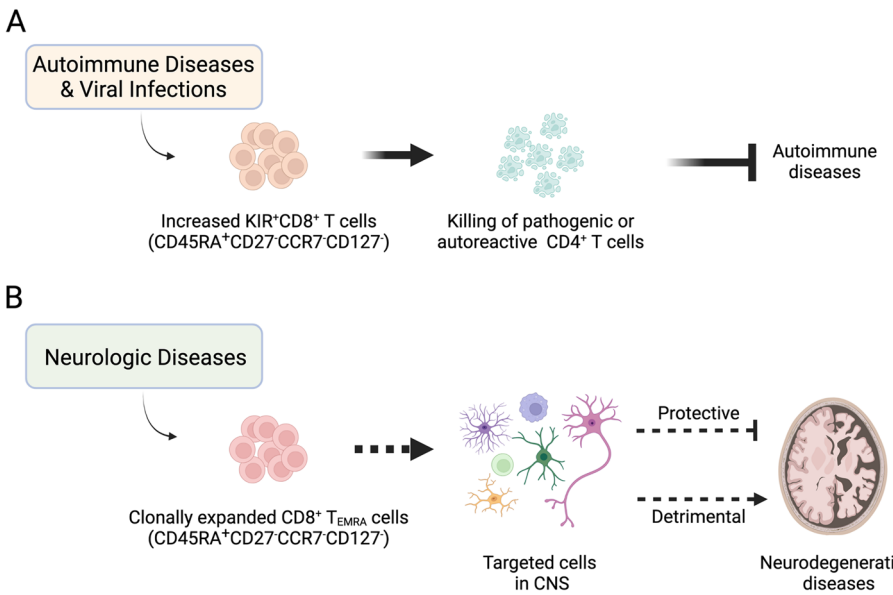

Fig. 1 $CD45RA^{+}CD27^{-}CCR7^{-}CD127^{-}$ $CD8^{+}$ T_{EMRA} cells in human diseases. **A** In human autoimmune diseases such as multiple sclerosis and during viral infection as such COVID-19, $CD45RA^{+}CD27^{-}CCR7^{-}CD127^{-}$ $KIR^{+}CD8^{+}$ T cells are increased in the peripheral blood and inflamed tissues of patients. $KIR^{+}CD8^{+}$ T cells kill T-cell receptor activated pathogenic and autoreactive $CD4^{+}$ T cells to prevent the development of autoimmune diseases and dampen autoimmune immune responses. **B** $CD45RA^{+}CD27^{-}CCR7^{-}CD127^{-}$ $CD8^{+}$ T_{EMRA} cells are clonally expanded in the cerebrospinal fluid from patients with neurodegenerative diseases such as multiple sclerosis (an autoimmune disease) and Alzheimer's disease. The function and specific cell-type classification of these $CD8^{+}$ T_{EMRA} cells are unknown. It is yet to be determined if they act like cytotoxic T lymphocytes to damage the center nervous system, or regulatory T cells to subdue rogue immune responses. Created with BioRender (Biorender.com)

differentiation directions, with one expressing high levels of killer-like receptors (KLRs) and killer cell immunoglobulin-like receptors (KIRs) [5], raising the possibility of that some of the clonally expanded $CD8^{+}$ T cells in neurologic diseases are immune suppressive $KIR^{+}CD8^{+}$ T cells (Fig. 1). Similar analysis can also be carried out with the single cell multi-omic datasets on CSF cells from patients with AD or MS to explore the diversity of $CD8^{+}$ T_{EMRA} cells. $CD161^{-}CD56^{+}$ $CD8^{+}$ Tregs were identified based on functional characterization of cloned $CD8^{+}$ T cells [11, 12]. Cloning of $CD8^{+}$ T_{EMRA} cells from the CSF followed by functional analysis in conjunction with global transcriptomic analysis may be a practical approach to define their diversity and unique molecular markers, and to understand their roles in neurodegeneration.

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Declarations

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