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Cytotoxic T Lymphocytes and Their Dual Role in Modulating Blood-Brain Barrier Integrity in Immune-Mediated Neurological Pathologies

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Abstract

The blood-brain barrier (BBB) is a dynamic, multicellular interface that preserves central nervous system (CNS) homeostasis by restricting entry of pathogens and circulating cells. Cytotoxic T lymphocytes (CTLs), comprising both $CD8^+$ and $CD4^+$ subsets, are central to adaptive immunity through targeted elimination of infected or transformed cells. However, in immune-mediated neurological disorders, including viral encephalitis, multiple sclerosis, Parkinson's disease, and glioma, CTLs effector functions can inadvertently compromise BBB integrity. Here, we integrate findings from primary research to delineate three principal mechanisms by which CTLs modulate the BBB: (1) direct cytotoxicity, in which perforin/granzyme release and FasL-Fas interactions induce endothelial cell apoptosis; (2) proinflammatory cytokine signaling, notably IFN-γ and TNF-α activation of JAK/STAT and NF-κB pathways in brain microvascular endothelial cells; and (3) chemokine-driven leukocyte trafficking, wherein CXCL10 and CCL5 gradients promote CTLs and bystander immune cell migration across the barrier. We further review evidence from *in vitro* and *in vivo* models that illustrate both protective and deleterious roles of CTLs at the neurovascular interface. By clearly specifying these mechanisms and their disease-specific contexts, this review establishes a unified framework for future investigations aimed at preserving BBB function while maintaining effective CTL-mediated immunity.

Key words

$CD8^+$ CTLs, $CD4^+$ CTLs, BBB, Neurodegenerative disease, Glioma, Infectious neurological disorder

1. Introduction

The blood-brain barrier (BBB) is a protective membrane that shields the central nervous system (CNS) from blood-borne toxins and pathogens, thereby preserving CNS homeostasis [1]. BBB dysfunction is a common pathological feature in many neurological diseases. Compromised BBB integrity or impaired function can significantly contribute to the progression of these conditions. In numerous neurological disorders, BBB disruption is frequently accompanied by an immune response within the nervous system. This response includes innate immunity, primarily neuroinflammation [2, 3], and adaptive immunity involving T and B cells [4-6]. Current research focuses primarily on T cell immunity in adaptive responses, as both innate and adaptive responses are essential for maintaining BBB function.

The innate immune system rapidly and nonspecifically responds to foreign pathogens or damaged cells by recognizing pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) [7]. In contrast, the adaptive immune system is activated over a longer period, involving the precise activation of T lymphocytes and B lymphocytes that are highly specific for their targets [8]. In general, B lymphocyte immune function is primarily mediated by antibodies secreted by their differentiated plasma cells following interaction with soluble antigens binding to the B cell receptor (BCR) [9]. T cell immunity operates through cell-to-cell interactions when the T cell antigen receptor (TCR) complex encounters peptide antigens presented by antigen-presenting cells (APCs). APCs present antigens via major histocompatibility complex class I or II (MHC I and MHC II), interacting respectively with the main subsets of T cells, CD8-positive ($CD8^+$) and CD4-positive ($CD4^+$) T cells [10].

This review examines cytotoxic T lymphocytes (CTLs) as an example of how T lymphocyte-mediated acquired immunity regulates to BBB dysfunction and its mechanisms. Unlike other reviews that predominantly focus on the role of innate immunity, such as neuroinflammation, in BBB function, this study concentrates on CTLs, explaining their targeting mechanisms, actions, and involvement in BBB dysfunction in neurological disorders. Therefore, the findings of this paper enhance the foundational knowledge of T lymphocyte immunity and BBB-related research, and suggest future research directions.

2. BBB structure and basic function

The BBB serves as a regulated interface between the peripheral circulation and the central nervous system (CNS) [11]. Although its existence was first noted in 1885, the precise nature of the BBB remained a topic of debate well into the 20th century [12]. The detailed process of discovering and naming the BBB is summarized in Supplementary Table 1 and briefly described as follows: in 1885, Paul Ehrlich reported that the brain is isolated from the bloodstream [13]. Subsequently, Edwin Goldman, Ehrlich's student, demonstrated that when Evans blue dye was injected into the ventricles, only the brain and spinal cord were stained, while peripheral organs remained unstained [14]. In 1922, Lina Stern introduced the term "barrière hémato-encéphalique" in French, which was later translated to "blood-brain barrier" [15]. The BBB is a multicellular vascular structure composed of brain microvessel endothelial cells, pericytes, astrocytes, neurons, and microglial cells. Junctional complexes, including tight and adherens junctions, are present at intercellular junctions within the BBB and are crucial for maintaining its low permeability [16]. A brief summary of the main functions of these components is given in Supplementary Table 2.

The BBB forms a physical and metabolic barrier that separates the CNS from peripheral tissues, protecting the brain by maintaining a stable environment [17, 18]. However, it also restricts drug entry into the CNS, complicating the treatment of brain diseases such as neurodegenerative disorders and brain cancer [19, 20]. Numerous studies have elucidated the BBB's physiological functions, including brain protection. In addition to serving as a physical and metabolic barrier against harmful substances, the BBB maintains CNS homeostasis, facilitates the selective transport of nutrients, ions, and signaling molecules, and modulates neuroinflammatory response.[21-23]. Wu et al. (2023) have detailed the functions of the BBB and the role of each component in their comprehensive review [11].

3. $CD8^+$ CTLs

T lymphocytes are divided into two distinct functional subgroups: $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes. $CD4^+$ T cells are known as T helper cells (Th), whereas $CD8^+$ T cells are referred to as CTLs [24]. Generally, CTLs act as powerful defenders against viral infections or intracellular pathogens by regulating the secretion of perforin and proteases in target cells, which induce apoptosis [25]. $CD4^+$ T cells indirectly contribute to infection clearance by modulating the activity of other immune cells, such as macrophages, neutrophils, B cells, and $CD8^+$ T cells [24]. However, pre-clinical and clinical studies have demonstrated that $CD4^+$ T cells possess cytotoxic programs and can directly kill cancer cells. Additionally, the cytotoxic function of $CD4^+$ T cells has been observed in other diseases, such as infections and autoimmune disorders [26-28]. In this section, we primarily discuss the production and activation of CTLs, as well as the mechanisms by which CTLs kill target cells.

Although $CD8^+$ and $CD4^+$ T lymphocytes represent the principal

effector subsets highlighted in this review, emerging evidence underscores the essential contribution of additional T cell subsets, notably regulatory T cells (Tregs) characterized by the $CD4^+CD25^+FOXP3^+$ phenotype [29, 30]. These cells are instrumental in preserving immune homeostasis and curbing excessive neuroinflammation [29]. By suppressing autoreactive T cell activity, Tregs facilitate peripheral immune tolerance [31, 32] and may secondarily modulate the structural and functional integrity of the BBB.

3.1 The differentiation of T cells

CTLs differentiation occurs in three distinct stages based on their sites of action. The first stage takes place in the red bone marrow, where common lymphoid progenitor cells differentiate into immature precursor T cells. Due to their high migratory capacity, these precursor T cells enter the circulatory system. Chemotactic agents or thymic factors from the thymus (such as thymotaxin, thymosin, and thymopoietin) direct their migration to the thymus, marking the second stage (circulatory system) and the third stage (thymus) of differentiation. In the thymus, the essential differentiation process involves thymic cells presenting CD- and TCR-positive T cells to MHC I and MHC II molecules to evaluate T-cell reactivity and direct their maturation pathways. T cells with TCR affinity for MHC I become $CD8^+$ T cells, whereas those with TCR affinity for MHC II become $CD4^+$ T cells [24]. Depending on cytokine and stromal cell signaling, they may further differentiate into T-helper and T-regulatory cells, both of which are subsets of $CD4^+$ T cells [33, 34]. The aforementioned process is illustrated in Fig. 1.

Tregs, characterized by high expression of CD25 and the transcription factor FOXP3 on $CD4^+$ T cells, are indispensable for maintaining peripheral immune tolerance and suppressing

autoimmunity [29, 30]. They exert immunosuppressive effects through both direct cell–cell interactions and the secretion of anti-inflammatory cytokines, including interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β) [35]. In addition to Tregs, other T cell subsets, such as T helper 17 (Th17) cells and γδ T cells, also participate in the regulation of neuroinflammation via distinct cytokine signatures and differential tissue-homing capacities [36, 37]. Increasing evidence indicates that Tregs contribute to the preservation of BBB integrity by attenuating proinflammatory cytokine production and promoting the stabilization of endothelial tight junctions in CNS autoimmune disease models [38, 39].

3.2 The activation of CD8$^+$ CTLs

The activation of CD8$^+$ CTLs is initiated through their initial interactions with target cells. Three critical components in this process are APCs, as well as the TCR and CD28 on CTLs.

APCs are essential in mediating interactions between T cells and their targets. Initially, APCs bind to target substances such as cancer cells, pathogens, viruses and others. Through phagocytosis and the action of proteases, these targets are degraded into antigenic peptide fragments, forming the MHC I -APC-target complex. CD8$^+$ T cells recognize the MHC I antigen peptide complex on this structure. Upon contact, T cells adhere to the complex and scan its surface. By homing towards chemokine and integrin gradients on APCs or target cells, CD8$^+$ T cells form immunological synapses between their supramolecular activation complex and adhesion molecules, such as intercellular adhesion molecules, on the target cell surface [40, 41]. During immunological synapse formation, TCR and CD28 on CD8$^+$ T cells play critical roles. The TCR is a complex structure composed of the antigen-binding subunit (TCRαβ) non-covalently linked with three CD3 co-receptor signaling subunits (ζζ, CD3δε, and CD3γε)

[42]. The intracellular CD3 contains immunoreceptor tyrosine-based activation motifs (ITAMs), which are essential for linking intracellular tyrosine kinase functions [42]. Hence, the CD3-ITAM pathway in TCR is crucial for assembling and transmitting intracellular signals following surface recognition by TCR. After TCR is activated by the MHC I -APC-target complex, a separate co-stimulatory signal is required; otherwise, T cells will not fully activate, leading to inactivity or apoptosis. This additional signal comes from the CD28 receptor on $CD8^+$ T cells, which binds to CD80/B7.1 or CD86/B7.2 on APCs, promoting T cell proliferation and cytokine production, such as IL-2 [43]. The aforementioned process is illustrated in Fig. 2. During this process, CD28 induces multiple signaling pathways in T cells, such as the PI3K-AKT and NF-κB pathways, leading to increased Bcl-xL expression and enhanced T cell survival [44]. Additionally, CD28 signaling protects $CD8^+$ T cells from reacting to self-antigens, thereby reducing the risk of tissue damage and autoimmunity. A more detailed description of $CD8^+$ T cell activation can be found in the review published by Hans Raskov in 2021 [45].

3.3 The $CD8^+$ CTLs-mediated mechanism of target-cell death

Once activated, $CD8^+$ CTLs demonstrate their potent cytotoxic abilities. As reported in various studies, $CD8^+$ CTLs bind to the Fas receptor on the target cell via the Fas ligand (FASL) on their surface, activating the death domain within the target cell. This activation subsequently triggers caspases and nucleases, leading to the fragmentation of the target cell's DNA [46]. More importantly, the cytotoxic activity of $CD8^+$ CTLs primarily depends on the release of granules containing granzymes, perforin, cathepsin C, granulysin, and other effector molecules. These granules fuse with the target cell membrane, allowing the effector molecules to enter the target

cell and create pores in the endosomal membrane, resulting in cell destruction [47, 48]. These processes occur within the immunological synapse (IS) formed between the $CD8^+$ CTLs and the target cell [41]. In brief, $CD8^+$ T cells exhibit persistent motility when interacting with target cells, which facilitates pore formation in the target cell membrane [47]. This allows the release of cytotoxic granules containing granzymes, perforin, cathepsin C, and granulysin, which fuse with the target cell membrane to initiate cell death [47]. Alternatively, the target cell may internalize a complex of granulysin, perforin, and granzymes through endocytosis of the cytotoxic T-cell membrane [48]. Once internalized, perforin and granulysin create pores in the endosomal membrane, allowing granzymes to escape into the cytoplasm, where they trigger apoptosis [48].

The IS is the interface where $CD8^+$ CTLs engage with target cells, facilitating TCR-mediated signaling and secretory events. Similar to natural killer cells, the initiation of IS formation in CTLs involves two signals[49]: the absence of MHC I recognition (disinhibition) and a positive signal from germline-encoded activation receptors that bind to specific ligands on target cells, such as lectins or hemagglutinins. Once antigenic peptides are recognized by the TCR on CTLs, the IS is formed, triggering complex signaling cascades involving the TCR, CD28, and associated pathways. These cascades lead to the realignment of the Golgi complex and microtubule network, with the microtubule-organizing center repositioning towards the IS and microtubules extending towards the distal pole. Along these microtubule tracks, effector granules are transported to the IS for secretion [50]. The mechanism by which granules enter target cells is complex and involves multiple modifications to the target cell's plasma membrane. A critical factor in this process is the

accumulation of Orai $Ca2^+$ channels and the involvement of t-SNARE syntaxin11. The activation of Orai $Ca2^+$ channels occurs in conjunction with IP3/$Ca2^+$-dependent activation and the translocation of STIM proteins to the endoplasmic reticulum near the IS. These activated STIM proteins interact with Orai channels, forming the store-operated $Ca2^+$ release-activated $Ca2^+$ complex, which drives store-operated $Ca2^+$ entry [51-53]. The increase in cytosolic $Ca2^+$ concentration is further enhanced by adjacent mitochondria [54, 55], ensuring optimal synaptic activation [56, 57]. Concurrently, t-SNARE syntaxin11, essential for lysosomal granule fusion, relocates to the IS and integrates into the plasma membrane through a VAMP8-dependent mechanism [58, 59]. This coordination ensures the precise positioning of release machinery components. Additionally, further modifications to the target cell membrane involve interactions between proteins on the granules and the target membrane, such as Rab27/Munc13 and VAMP/Munc18. Although the specific details of these molecular mechanisms are extensively covered in various reviews [60], they are not elaborated on here. These interactions highlight the intricate regulation of granule fusion and release, which is crucial for the effective cytotoxic response of CTLs.

An overactivated $CD8^+$ CTLs response can be detrimental, leading to autoimmune disorders, rejection of transplanted cells, and graft-versus-host disease. This is because the lytic machinery of CTLs can mistakenly target self-tissues or host tissues [61]. To prevent such uncontrolled activation, immune checkpoint molecules, which are transiently expressed inhibitory receptors on the cell surface, are essential. They regulate $CD8^+$ CTLs activation, ensuring the immune response is properly modulated even in the presence of strong activation signals [62]. This checkpoint molecule is also present in

other immune cells, including natural killer cells and activated macrophages, where they perform similar regulatory functions. Key checkpoint molecules include programmed cell death receptor 1 (PD-1 or CD279), CTLA-4, lymphocyte-activation gene 3 (LAG-3), T-cell immunoglobulin and mucin domain-3 (TIM-3), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), and inducible T-cell co-stimulatory receptor (ICOS). The mechanisms by which these immune checkpoints function have been extensively reviewed [63, 64], and in this paper, their main modes of action are displayed in Supplementary Table 3. However, malignant tumor cells can exploit these inhibitory signals to evade the immune response and enhance their own survival [65].

The development of monoclonal antibodies targeting immune-inhibitory receptors, known as checkpoint inhibitors, represents a major breakthrough in immuno-oncology, significantly improving the clinical outcomes of various cancers [66]. This therapeutic approach enhances antitumor immune responses while also revitalizing exhausted $CD8^+$ T cells, thereby increasing tumor cell eradication. Among these therapies, anti-PD-1 agents have been particularly transformative in the treatment of metastatic melanoma, demonstrating remarkable clinical efficacy [67, 68]. Several checkpoint inhibitors targeting the PD-1 pathway have received approval in the United States, including three PD-1 inhibitors (pembrolizumab, nivolumab, and cemiplimab), and three PD-L1 inhibitors (atezolizumab, avelumab, and durvalumab). Current research focuses on improving the efficacy and reducing the toxicity of these agents by combining them with other therapeutic modalities, such as immunotherapies or cytotoxic chemotherapies. Notably, the combination of PD-1/PD-L1 inhibitors with CTLA-4 inhibitors has yielded promising clinical outcomes, as demonstrated by the

approval of nivolumab in combination with ipilimumab for the treatment of metastatic melanoma, advanced renal cell carcinoma, and mismatch repair-deficient colorectal cancer [69, 70].

4. CD4$^+$ CTLs

4.1 Ontogeny and Differentiation of CD4$^+$ CTLs

CD4$^+$ CTLs differentiate from naive CD4$^+$ T cells under conditions of persistent antigen stimulation and pro-inflammatory cytokines such as IL-2, IL-15 and IL-22 [71-73]. Transcription factors T-bet and Eomesodermin coordinate the acquisition of cytotoxic programs by upregulating perforin and granzyme B expression [73, 74]. Co-stimulatory signals via CD28 and 4-1BB further enhance CD4$^+$ CTL expansion and survival [75]. In chronic infections, such as tuberculosis, CD4$^+$ CTLs increase in frequency and partially restore pathogen clearance when CD8$^+$ CTLs exhibit an exhausted phenotype marked by PD-1 and TIM-3 upregulation [76, 77]. Similarly, in autoimmunity models, CD4$^+$ CTLs compensate for impaired CD8$^+$ responses by targeting MHC II-expressing antigen-presenting cells and sustaining local cytotoxicity [78].

4.2 Effector Mechanisms of CD4$^+$ CTLs

Conventional CD4$^+$ T cells, including thymus-derived FOXP3 regulatory T cells, are part of the Th cell lineage, characterized by a TCR that recognizes MHC II [79]. The functional diversity of Th subsets is further expanded by the presence of CD4$^+$ T cells with cytotoxic capabilities, known as CD4$^+$ CTLs. Initially, these CD4$^+$ CTLs were dismissed as artifacts from exhausted, long-term cultured T cell lines or miscategorized within the Th1 subset [80, 81]. However, research over the past decades has demonstrated that CD4$^+$ CTLs are a distinct Th subset with antigen-specific cytotoxic activity, observable in both humans and mice [82, 83].

CD4+ CTLs, similar to CD8+ T cells, utilize two primary effector mechanisms to eliminate target cells [84, 85]. The first involves the release of cytotoxic granules containing perforin and granzyme B, which induce perforin oligomerization and pore formation in the target cell membrane [86]. The second mechanism involves Fas/FasL-mediated apoptosis, where FasL on CD4+ CTLs binds to Fas receptors on target cells, activating Caspase 8 and subsequently Caspase 3, leading to apoptosis. Detailed descriptions of these mechanisms are provided in the "CD8+ CTLs" section of this paper. In contrast to CD8+ T cells, which recognize antigens presented by MHC I molecules, CD4+ CTLs recognize peptides presented by MHC II molecules on APCs. Therefore, it is unlikely that CD4+ CTLs simply substitute the function of CD8+ CTLs.

4.3 Compensatory Roles in Chronic Infection and Autoimmunity

The distinctive characteristic of CD4+ CTLs is their capacity to kill target cells, mirroring and complementing the cytotoxic function of CD8+ T cells. Although CD4+ CTLs are found in low numbers under normal conditions [86], their population increases significantly during chronic viral infections such as those caused by cytomegalovirus, dengue virus, ectromelia virus, lymphocytic choriomeningitis virus, and other pathogens [87-90]. Growing evidence suggests that the cytotoxic activities of CD4+ T cells against infected or transformed cells likely compensate for the reduced killing efficacy of exhausted CD8+ CTLs, which can be inhibited by virus-induced checkpoint molecules [91]. For instance, during chronic Mycobacterium tuberculosis (Mtb) infection, T-cell immunity is suboptimal due to the expression of inhibitory receptors like PD-1 and TIM-3, resulting in reduced cytokine production [76, 77]. Consequently, CD8+ T cells exhibit an exhausted phenotype, and

CD4+ T cells adopt a cytotoxic profile marked by the expression of Tbx21, potentially compensating for the impaired function of CD8+ T cells during active tuberculosis [92].

5. The role of CTLs in the regulation of BBB function

The association between the BBB and CTLs was first reported by Wyde et al. in 1983 [93], as recorded in the PubMed database. Wyde and colleagues compared the dissemination of a neurovirulent strain of influenza A/WSN (HON1) virus from infected lungs to brains of thymus-deficient nude and immunocompetent furred mice, both inoculated intranasally. Their results revealed that, in immunocompetent mice, the virus was typically cleared from the lungs of survivors, with minimal cases of viral spread to the brain. In contrast, nude mice exhibited frequent and early deaths, with significant viral titers in the brain and histological evidence of encephalitis. Notably, adoptive immunization of nude mice with CTLs, which had been stimulated *in vitro* 24 hours after intranasal challenge, led to a reduction in both brain virus titers and mortality [93]. These findings underscored the crucial role of T lymphocytes in inhibiting the dissemination of neurotropic viruses from the lungs to the brain.

Wyde's pioneering study suggested for the first time that T lymphocytes are integral to the BBB's defense against viral invasion. In the 1980s, Hafler and colleagues further examined and reviewed the role of T cells in multiple sclerosis and other inflammatory central nervous system diseases [94]. For instance, Hafler et al. initiated clinical trials using anti-T-cell murine monoclonal antibodies (MAbs) to treat multiple sclerosis, aiming to develop a targeted and non-toxic immunotherapy [95]. During infusions with anti-T11, a pan-T-cell monoclonal antibody targeting the CD2 receptor, they observed that the antibody bound to peripheral blood

T cells without inducing significant cell lysis, and did not immediately modulate the CD2 surface structure. Additionally, they found that the BBB remained relatively impermeable to the antibody. This unique scenario allowed researchers to study the migration of peripheral T cells into the CNS in patients with progressive multiple sclerosis.

Following these groundbreaking studies, researchers began investigating how CTLs contribute to neurological dysfunction, particularly by crossing or disrupting the BBB. In this context, we focus on the role of CTLs in maintaining the integrity of the BBB and their associated functions in neurological conditions, particularly brain tumors, non-tumor neurological diseases such as multiple sclerosis and Parkinson's disease, as well as virus-induced or pathogen-induced neurological disorders.

5.1 Brain-related tumors

Brain metastases of tumors

The association between CTLs and BBB in brain tumor models was initially reported by Gordon et al. using a P511 mastocytoma cell tumor model [96]. Their research demonstrated that, on the seventh day following cannula implantation in the cerebral cortex, brain tumors developed while the BBB remained intact. Importantly, the population of P511-specific non-cytolytic CTL precursors (pCTLs) were identified at the brain tumor site, suggesting that these pCTLs, generated in the periphery, migrated to the brain tumor area. The incomplete activation of these cells, likely due to the inhibitory microenvironment of the central nervous system, indicated that the unique structure of the BBB prevents their full activation, thus reducing their cytotoxic potential. Furthermore, when the tumor cells were injected at a flank site, similar phenomena were observed in the brain metastasis model of P511 mastocytoma cells [96].

Glioma

Glioblastoma multiforme (GBM) is the most common and aggressive malignant primary brain tumor in adults. Focused ultrasound (FUS) can temporally and locally open the BBB. In a GBM mouse model, Chen et al. utilized FUS to disrupt the BBB, leading to significant changes in tumor-infiltrating lymphocyte (TIL) populations within the brain, particularly increasing the number of $CD3^+CD8^+$ CTLs in the tumor region. This resulted in notable inhibition of tumor progression and improved survival rates in the animals [97]. Oncolytic virotherapy is another promising approach to improve the poor prognosis of malignant brain tumors. The rat H-1 parvovirus (H-1PV) has shown tumor suppression in preclinical glioma models through direct oncolysis and stimulation of anti-cancer immune responses [98, 99]. Because the virus can penetrate the blood-brain/tumor barrier and spread extensively within the tumor, significant changes were observed in the tumor microenvironment upon viral infection. These changes included microglia/macrophage activation and CTLs infiltration, indicating that H-1PV may trigger an immunogenic response [98, 99]. Numerous similar studies have reported other methods and vectors capable of altering the brain's immune microenvironment, such as the RNA-modification of T Cells, modified nanoparticles, and others [100-104]. These approaches must successfully penetrate the BBB—a major challenge in brain cancer treatment—and increase CTLs infiltration at the tumor site. Notably, the increased CTLs are predominantly CD8 positive [100-104]. Thus, current research on brain tumors, CTLs, and the BBB primarily seeks methods to cross the BBB and enhance the cytotoxic function of immune cells, such as $CD8^+$ CTLs, at the tumor site. However, there is no research on the direct effects of CTLs on the BBB in brain tumors.

5.2 Non-neoplastic neurological diseases or dysfunctions

Multiple sclerosis (MS)

MS is a central nervous system disease characterized by inflammation and autoimmunity. In 1993, researchers discovered that peripheral T cells from patients with acute MS exhibit a cytotoxic effect on brain endothelial cells [105]. This observation indicates that T cell-induced cytotoxicity towards brain endothelial cells might play a role in increasing BBB permeability and triggering immune responses in acute MS [105].

The Theiler's murine encephalomyelitis virus (TMEV) model is a key tool for studying MS. Researchers have used this model to explore the role of CTLs in MS, with significant contributions from Georgette L. Suidan's team between 2008 and 2012 [106-108]. They found that $CD8^+$ CTLs might disrupt the BBB through mechanisms involving perforin and vascular endothelial growth factor (VEGF). Their research suggested that, unlike their typical cytotoxic role against harmful cells, $CD8^+$ CTLs use a non-apoptotic perforin-dependent mechanism to break down BBB tight junctions. This mechanism involves the activation of astrocytes, alteration of BBB tight junction proteins, and increased CNS vascular permeability [106]. Another pathway includes VEGF, where $CD8^+$ CTLs interact with neurons, either directly or indirectly through other immune cells, leading to VEGF upregulation, which disrupts tight junctions and increases vascular permeability [107, 108].

Researchers have also studied the relationship between CTLs and the BBB in MS, particularly focusing on the ability of CTLs to penetrate the BBB. Studies have shown that in MS, B cell-derived interleukin-15 (IL-15) increases the proportion of $CD8^+$ CTLs in the brain and enhances their ability to cross the BBB. However, the molecular mechanisms by which IL-15 facilitates $CD8^+$ CTLs

migration across the BBB remain unclear [109]. Other researchers hypothesize that this process may involve microRNAs of CTLs or P-glycoprotein in brain endothelial cells [110]. Aya A. Elkhodiry found a significant correlation between the downregulation of microRNA-155 in $CD8^+$ CTLs isolated from MS patients' blood samples and the upregulation of intracellular adhesion molecule 1 (ICAM1) and integrin subunit beta 2 (ITGB2), both of which are critical for migration through the BBB [110]. Similarly, Gijs Kooij's 2014 study demonstrated that endothelial P-glycoprotein mediates the migration of $CD8^+$ CTLs across the BBB [111]. Their research showed that reducing P-glycoprotein expression in endothelial cells using shRNA significantly decreased the transendothelial migration and adhesion capabilities of $CD8^+$ and $CD4^+$ CTLs in an *in vitro* BBB model. This finding was further corroborated *in vivo* using cell-specific CCL2 knockout mice, revealing that P-glycoprotein regulates $CD8^+$ T cell migration via CCL2 secretion [111].

Additionally, $CD4^+$ CTLs have been reported to play a crucial role in MS. These $CD4^+$ T cells co-express NKG2D, an activating receptor predominantly expressed on NK cells, $CD8^+$ T cells, and γδ T cells in humans and mice [112]. Tobias Ruck et al. reported that these $CD4^+$ $NKG2D^+$ T cells exhibit high levels of migration, activation, and cytolytic activity. In an *in vitro* BBB model, NKG2D facilitated the migration of $CD4^+$ $NKG2D^+$ cells through endothelial cells [113].

Parkinson's disease

In Parkinson's disease (PD), a progressive neurodegenerative disorder affecting 2–3% of the population over 65 years old [114], peripheral $CD4^+$ CTLs have been also reported to regulate BBB dysfunction. In 2023, Shi et al. used single-cell RNA sequencing to elucidate the potential mechanisms by which $CD4^+$ T cells contribute to BBB disruption [115]. Their study revealed a significant increase

in the proportion of PD-related $CD4^+$ CTLs in the peripheral blood mononuclear cells of PD patients. Moreover, these $CD4^+$ CTLs exhibited significantly elevated expression of the *Ifng* gene, which is particularly sensitive to endothelial cells compared to other midbrain cell types. Further cell-cell communication analysis identified that during the process of $CD4^+$ CTLs weakening endothelial cell tight junctions, IFNG/IFNGR1 and SPP1/ITGB1 were the primary signaling pathways between CTLs and endothelial cells [115].

Epilepsy

In epilepsy research, direct evidence of CTLs regulating BBB function is currently lacking, but several studies have explored related functional aspects. Nicola Marchi and colleagues conducted a study using splenectomy to immunosuppress rats, which reduced various immune cells, including CTLs, and subsequently decreased mortality in a pilocarpine-induced rat epilepsy model [116]. Furthermore, they induced epilepsy in perforin-deficient mice with pilocarpine and observed reduced BBB damage compared to controls [116]. Since perforin is a key effector molecule for CTL-mediated cytotoxicity, this study indirectly supports the idea that CTL-perforin pathways contribute to BBB damage [116], similar to findings by Suidan's team in the TMEV model [117]. Another study examined the effects of rapamycin (RAP) on CTLs and BBB in epilepsy [118]. This research reported that RAP increased the levels of total T cells ($CD3^+/CD45^+$) and T helper cells ($CD3^+/CD4^+$) in epileptic rats while reducing the levels of CTLs ($CD3^+/CD8^+$). Simultaneously, harmful BBB factors such as MMP-9, MMP-2, and inflammatory cytokines were decreased [118]. This study highlighted an inverse relationship between BBB function and CTLs

554 in an epilepsy model but did not further analyze the underlying
555 mechanisms or provide detailed correlations.

556 ***Hemorrhagic stroke***

557 In hemorrhagic stroke, CCL5 in astrocytes has been shown to play a
558 critical role in the interaction between peripheral CTLs and
559 astrocytes, leading to BBB disruption. Zhou et al. identified CCL5 as
560 one of the top upregulated genes in RNA sequencing results from
561 astrocytes activated by IL-1α, TNF-α, and complement component
562 1q treatment [119]. Functional validation demonstrated that
563 knocking out CCL5 in astrocytes reduced $CD8^+$ T cell infiltration into
564 the brain, but did not affect the infiltration of $CD4^+$ T cells and
565 myeloid cells. Moreover, reduced CCL5 expression decreased BBB
566 disruption following hemorrhagic stroke, although this protective
567 effect was nullified by the supplementation of $CD8^+$ CTLs [119].

568 ***Susac syndrome***

569 Susac syndrome (SuS) is a rare neuroinflammatory disease
570 characterized by endothelial dysfunction in the central nervous
571 system, manifesting as focal microangiopathy that affects the small-
572 to-medium-sized vessels of the brain, retina, and inner ear [120, 121].
573 The pathogenesis of SuS remains highly controversial, with the most
574 widely accepted theory suggesting an autoimmune process [122]. In
575 a 2019 publication, Catharina C. Gross and colleagues proposed that
576 SuS is an endothelial injury disease driven by CTLs targeting an
577 unknown antigen [123]. Specifically, an unidentified antigen
578 activates $CD8^+$ CTLs, enabling them to secrete granzyme B and
579 perforin. These activated CTLs then accumulate in the
580 microvasculature of the brain, retina, and inner ear, adhere to
581 endothelial cells, and induce apoptosis via granzyme B and perforin,
582 thereby disrupting the BBB and causing localized microhemorrhages.
583 This initiates a cascade of neuroinflammation, leading to the loss of

astrocytes, oligodendrocytes, neurons, and axons. Eventually, ischemic lesions infiltrate surrounding astrocytes, transforming into gliosis [123]. Throughout the disease progression, the granzyme B and perforin-dependent damage by $CD8^+$ CTLs to endothelial cells and the BBB is a critical process. Understanding the activation mechanisms of $CD8^+$ CTLs is crucial for advancing the treatment and prevention of Susac syndrome.

In 2023, Carmen Gonzalez-Fierro further validated Gross's hypothesis using an *in vitro* co-culture model of primary brain microvascular endothelial cells and $CD8^+$ CTLs [124]. This study confirmed that perforin-dependent cytotoxicity is a key mediator of endothelial cell death, suggesting this mechanism as a foundational aspect of SuS pathogenesis [124].

Schizophrenia

N. Müller examined the expression of adhesion molecule receptors, specifically VLA-4 and LFA-1, on Th ($CD4^+$) and T suppressor/cytotoxic ($CD8^+$) lymphocytes in patients with schizophrenia, both before and during antipsychotic treatment [125]. The investigation revealed that the proportion of VLA-4^+/$CD4^+$ and VLA-4^+/$CD8^+$ cells increased significantly during antipsychotic therapy. Furthermore, VLA-4^+/$CD4^+$ and LFA-1^+/$CD4^+$ cells were strongly linked to disturbances in the BBB [125]. Since this study was conducted in the late 20th century, the researchers did not validate these correlations or delve into the underlying mechanisms comprehensively.

5.3 Virus-induced or pathogen-induced neurological disorders

Cerebral malaria

Cerebral malaria, a severe complication of Plasmodium falciparum infection, involves associations between CTLs and BBB similar to

those seen in neurological diseases like SuS and MS [106, 123]. In cerebral malaria, $CD8^+$ T lymphocytes induce endothelial cell apoptosis through a perforin-dependent mechanism, contributing to the observed lethality in murine models [126, 127]. Researchers have explored strategies to mitigate CTLs toxicity to the BBB in experimental malaria, such as modulating the functions of antigen-presenting cells and controlling the migration of activated T cells [128-131]. Johanna F. Scheunemann has comprehensively reviewed these findings [132]; thus, further elaboration is unnecessary here.

Human T-cell leukaemia virus 1

Human T-cell leukemia virus type 1 (HTLV-1) infection can lead to T-cell leukemia and inflammatory diseases, most notably HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [133]. In TSP/HAM, HTLV-1-infected T cells, anti-HTLV-1 cytotoxic T cells, and macrophages infiltrate the cerebrospinal fluid, indicating that the disease involves disruption of the blood-brain barrier (BBB) [134]. Nirit Mor-Vaknin, in 1998, demonstrated that HTLV-1-infected T cells can fuse with and damage astrocytes *in vitro*, proposing that the destruction of astrocytes by HTLV-1-infected T cells leads to BBB disruption [134]. Furthermore, research by Guangyong Ma has shown that peripheral HTLV-1-infected T cells can transfer HTLV-1 to brain endothelial cells, causing BBB damage [135]. Thus, peripheral T-cell-mediated viral transmission may be a key mechanism in HTLV-1-induced BBB disruption.

Dengue virus

In acute viral encephalitis induced by Dengue virus (DENV) infection, $CD8^+$ CTLs likely play a major role. Tsung-Ting Tsai and colleagues found that in DENV-infected mice [136], $CD8^+$ CTLs infiltration into the central nervous system resulted in CNS inflammation and BBB disruption. During this process, microglial cells exhibited significant

antigen-presenting cell functions, stimulating CTLs proliferation and activation. Conversely, depleting microglial cells eliminated DENV-induced antiviral cytokine expression and $CD8^+$ CTLs infiltration, restoring BBB integrity and neurological function [136].

Lymphocytic choroid plexus meningitis virus

Lymphocytic choriomeningitis virus (LCMV) infection in mice causes fatal immunopathology and convulsive seizures through BBB disruption [137, 138]. LCMV-specific CTLs are crucial in this process. Jiyun V. Kim and colleagues reported that during acute viral meningitis, activated $CD8^+$ CTLs not only damage the BBB through downstream effector molecules (e.g., IFN-γ receptor, TNF-α, Fas, granzyme, perforin) but also express various chemokines that recruit bone marrow mononuclear cells responsible for vascular injury [139].

Adeno-associated virus (AAV)

AAV, a member of the *Parvoviridae* family, is widely used in scientific research. Although intracranial microinjection of AAV is generally regarded as a safe and effective method for inducing transgene expression in the central nervous system, high doses of AAV can exhibit neurotoxicity and damage the BBB. This damage may be mediated by the infiltration of peripheral CTLs into the CNS. This hypothesis is supported by findings that neuronal loss induced by high-dose AAV injection can be alleviated by depleting infiltrating T immune cells [140].

5.4 Advanced Experimental Models to Elucidate CTL–BBB Dynamics

Recent technological innovations have significantly enhanced our ability to dissect CTLs interactions with the BBB under near-physiological conditions. These models span high-resolution single-cell omics, intravital microscopy, and biomimetic

"BBB-on-a-chip" platforms, each offering unique insights into CTLs trafficking, signaling, and barrier disruption.

Single-cell Omics

Yan et al. applied droplet-based single-cell RNA sequencing to isolate and profile over 33,000 $CD4^+$ CTLs from both peripheral blood and CNS infiltrates of Parkinson's disease patients [115]. They discovered pronounced upregulation of IFNG and SPP1 in CTLs, accompanied by elevated IFNGR1 and ITGB1 expression in brain microvascular endothelial cells—identifying a pathogenic signaling axis that undermines tight junction integrity. Complementarily, Patil et al. performed single-cell transcriptomics on peripheral blood mononuclear cells (PBMCs) from healthy donors, delineating $CD4^+$ CTL differentiation trajectories marked by sequential induction of cytolytic effectors GZMB and PRF1 [88].

Intravital Imaging

Kim et al. and Phillip et al. utilized two-photon intravital microscopy in lymphocytic choriomeningitis virus (LCMV)–infected mice to visualize CTL behavior within intact brain microvasculature [139, 141]. Their studies reveal CTL crawling, arrest, and transendothelial migration guided by chemokine gradients (e.g., CXCL10), correlating precisely with localized BBB permeability increases.

Human BBB-on-a-Chip Models

Nair et al. engineered a microfluidic BBB model comprising human brain microvascular endothelial cells cultured against an extracellular matrix gel within 40 parallel channels [142]. Upon exposure to TNF-α and IL-1β, transendothelial electrical resistance (TEER) declined by ~30%, and adhesion molecule expression (ICAM-1, VCAM-1) increased. When primary human T cells were perfused under flow along a CXCL12 gradient, they faithfully recapitulated inflammation-driven extravasation observed *in vivo*.

By bridging reductionist and *in vivo* approaches, these advanced models afford unprecedented mechanistic resolution of CTL–BBB dynamics. Single-cell omics elucidate the molecular programs within individual CTLs and endothelial cells; intravital imaging captures real-time cellular behavior within the native microenvironment; and BBB-on-a-chip platforms provide scalable, human-relevant systems for high-throughput interrogation of immune cell transmigration. Collectively, these methodologies pave the way for targeted interventions that preserve barrier integrity while modulating neuroimmune crosstalk.

5.5 Translational Caveats and Data Gaps

While murine models have elucidated key mechanisms of CTL–BBB modulation, their direct extrapolation to human disease is constrained by several factors:

Species and model differences

Rodent and human brain microvascular endothelial cells differ markedly in tight junction composition (e.g., claudin-5 levels [143]) and transporter expression (P-glycoprotein, BCRP [144]), altering permeability and leukocyte trafficking.

Temporal dynamics

Experimental antigen challenges in mice typically unfold over hours to days, whereas human neurodegenerative and autoimmune disorders feature chronic, low-grade inflammation persisting for months to years. Such divergence may obscure the progressive BBB remodeling observed clinically.

Genetic homogeneity vs. diversity

Inbred mouse strains lack the genetic polymorphisms present in human populations (e.g., cytokine and chemokine receptor variants) [145] that critically shape CTL responses and barrier interactions.

Clinical data scarcity

Few studies have quantified CTL infiltration or BBB integrity in human CNS tissues. MRI and PET assessments of barrier leakage remain limited to small cohorts in multiple sclerosis [146] and post-COVID syndromes [147], whereas, post-mortem immunohistochemical analyses of CTLs are rare.

Underutilized Human In Vitro Models

Although induced pluripotent stem cell (iPSC)–derived BBB organoids and microfluidic "BBB-on-a-chip" platforms can recapitulate shear stress and multicellular architecture [142, 148], they are not yet widely adopted for investigating CTL transmigration. Addressing these gaps will demand integration of humanized animal models, longitudinal patient sampling, advanced in vivo imaging tools, and broader deployment of human BBB platforms to ensure that preclinical insights align with human pathophysiology.

6. Therapeutic Implications and Future Strategies

Translating mechanistic insights into effective therapies requires approaches that precisely modulate CTL activity at the BBB while preserving barrier integrity:

Immune Checkpoint Blockade

Agents such as anti–PD-1/PD-L1 antibodies (e.g., nivolumab) can rejuvenate exhausted CTLs [149, 150] but may aggravate BBB permeability through enhanced cytokine release.

Chemokine-axis Blockade

Targeting chemokine receptors (e.g., CXCR3 antagonists) reduces CTL recruitment and BBB disruption in experimental autoimmune encephalomyelitis [151, 152], while the CCL5-CCR5 axis has demonstrated efficacy in hemorrhagic stroke models [153].

Localized BBB Modulation

Focused ultrasound-mediated BBB opening permits site-specific delivery of immunomodulators, as shown in glioma with enhanced

CTL infiltration [154, 155]. Receptor-targeted nanoparticles (e.g., Angiopep-2-decorated carriers co-delivering granzyme B and CpG) further concentrate CTL-directed agents at the neurovascular interface [156].

CTLs Cytotoxicity Attenuation

Small-molecule inhibitors of perforin and granzyme (e.g., compounds described by Gonzalez Fierro et al., 2023 [124]) selectively dampen CTL-mediated endothelial apoptosis, offering potential adjunctive therapy in Susac's syndrome and multiple sclerosis.

Integrating these therapeutic avenues within humanized platforms will be essential to achieve durable neuroprotection alongside robust pathogen or tumor clearance.

7. Conclusion and further challenges

CTLs exert profound effects on BBB integrity in immune-mediated neurological disorders, including autoimmune diseases and pathogen-induced conditions. Three principal mechanisms have been identified (Fig. 3): a. Direct cytotoxicity, wherein CTLs deploy perforin and granzyme to induce endothelial apoptosis [157]; b. Neuron-mediated disruption, via CTL-altered neuronal VEGF production that compromises tight junctions [107]; and c. Immune-cell facilitation, whereby other leukocytes or resident glia amplify CTL-triggered BBB damage [108, 139]. Additional context-specific pathways, such as HTLV-1 vesicular transmission by CTLs, underscore the complexity of CTL–BBB interactions [134].

To integrate the diverse molecular mechanisms detailed above, we propose a unified model comprising three interlinked axes by which CTLs disrupt BBB integrity: a. Perforin/Granzyme Cytotoxicity: CTLs release perforin and granzyme B, forming pores in endothelial membranes and activating caspase cascades to induce apoptosis. b.

IFN-γ/TNF-α Signaling: CTL-derived IFN-γ and TNF-α activate JAK/STAT and NF-κB pathways in brain microvascular endothelial cells, downregulating tight junction proteins. c. Chemokine-Mediated Trafficking: CTLs secrete CXCL10 and CCL5, establishing chemotactic gradients that recruit additional immune cells via CXCR3 and CCR5, promoting diapedesis. These axes converge synergistically to amplify BBB permeability, suggesting that combinatorial therapeutic strategies targeting multiple pathways may enhance barrier preservation.

Despite the beneficial role of activated CTLs, particularly $CD8^+$ cells, in targeting pathogens and infected cells in the brain, their potent cytotoxicity often results in collateral damage to healthy cells. Perforin, a major toxic factor, can inadvertently harm normal cells, disrupting the BBB structure, which is primarily composed of brain endothelial cells. Peripheral CTLs must traverse this natural barrier to exert their pathogen-killing function within the brain. Thus, CTL toxicity towards endothelial cells is partly aimed at facilitating brain entry, but this breach can lead to neurological dysfunction. In autoimmune diseases, activated peripheral CTLs also congregate around brain endothelial cells, causing BBB damage and neurological disorders. This is partly due to increased MHC I expression on endothelial cells, which may attract $CD8^+$ CTLs [157]. Granzyme B and perforin are primary toxic mediators for CTLs. Research shows that reducing or knocking out perforin expression in mouse disease models protects BBB integrity, improves disease symptoms, and increases survival rates. Therefore, CTLs might be more harmful than beneficial in certain disease stages, and reduced perforin expression could protect the BBB and enhance survival. However, determining when to inhibit or enhance CTLs function requires further investigation.

CD4$^+$ CTLs, although less studied, similarly perturb BBB function. We hypothesize that these cells predominantly assist immune responses under homeostatic conditions and may employ non perforin pathways, such as IFN γ/IFNGR1 and SPP1/ITGB1 signaling, to exert cytotoxicity during chronic inflammation. Rigorous validation of these mechanisms is warranted.

The ongoing global COVID-19 pandemic, caused by SARS-CoV-2, persists despite advancements in vaccination and increased natural immunity. Prolonged infection has been linked to brain fog and cognitive impairment, with disruption of the BBB playing a critical role [158, 159]. Research has shown that SARS-CoV-2 infection triggers CD3$^+$ T cell infiltration in the hippocampus and brainstem of infected mice [160]. Transcriptomic sequencing of peripheral blood mononuclear cells from COVID-19 patients with cognitive dysfunction also revealed significant enrichment of pathways related to T cell differentiation and activation, as identified through Gene Ontology (GO) analysis [161]. These findings suggest a potential role for T cells, including CTLs, in regulating BBB function during SARS-CoV-2 infection. However, the direct involvement of CTLs and the underlying mechanisms require further investigation.

Collectively, CTLs are pivotal regulators of neurovascular integrity. Future research must integrate high-resolution *in vivo* imaging, humanized BBB platforms, and single-cell omics to map CTL dynamics and identify targets for selective modulation, thereby preserving barrier function without compromising host defense.

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Author contributions

B.L. for writing original draft, funding acquisition, production of the figures, and tables, writing - review & editing. B.L. and P.L. for investigation and production of the figures, and tables, checking all figures and tables. W. X. and B.L. for discussing revision strategies of manuscript, writing - review & editing. All authors contributed to the discussion.

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Declarations

Ethics statement

Not application

Consent for publication

All authors read and approved to publish this manuscript.

Competing interests

The authors declare that they have no competing interests.

876 **Supplementary Table 1 The discovery process of the BBB**

Name	Year	Contribution	Reference
Ridley Humphrey	1695	The low permeability of small cerebral vessels	[162]
Ehrlich Paul	1885	The isolating between brain and bloodstream	[13]
Lewandowsky Max	1909	Terming this new concept as a German name bluthirnschranke	[163]
Goldmann Edwin Ellen	1909	Only the brain and the spinal cord can be stained by Evans blue injected in ventricles	[14]
Stern Lina & Raymond Gautier.	1921	Naming it as “barrière hémato-encéphalique” in French, and then translated into BBB	[15]
Stern Lina	1929	the BBB was not mature during embryogenesis	[164]

878 **Supplementary Table 2 The main functions of components of BBB**

Components	Functions	Reference
Endothelial cells	Endothelial cells are tightly interconnected, forming distinct lumenal and abluminal membrane compartments	[165]
Pericytes	Pericytes are embedded in the basement membrane and lie abluminal to the endothelial cells, and in close communicate with endothelial cells	[166, 167]
Astrocytes	Astrocytes surround blood vessels in the brain, serving as the interface between neurons and endothelial cells	[168]
Tight junctions	Tight junctions reside between endothelial cells, serving as the main functional components in sustaining the permeability barrier and controlling tissue homeostasis	[169]
Adherent junctions	Adherent junctions are fundamental for the integrity of BBB, any change of adherens junctions may disrupt inter-endothelial cell connections	[170]

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Supplementary Table 3 The main functions of immune checkpoints

Checkpoints	Functions	Reference
PD-1	Binding with its ligand PD-L1/PD-L2 of target cells, counteracting CD80–CD28 signaling transduction of CTLs.	[171]
CTLA-4	Interferes with CD8 T-cell movements and the ability to form stable conjugates with APCs, thus reducing the contact time between cells	[172]
LAG-3	Binding with CD3 in the TCR complex and inhibiting its signal transduction, leading to reduced T cell proliferation and cytokine production	[173]
TIM-3	The switching of the binding TIM-3 and Bat3 or Fyn, further inhibiting upstream TCR signaling	[174]
TIGIT	Inhibiting TCR signaling by binding with CD155 of APCs	[175]
ICOS	Weaking the function of CD28 signaling by binding with CD275 of APCs	[176]

Fig. 1 Schematic representation of the differentiation of T cells from common lymphoid progenitors.

Schematic representation of the differentiation of T cells from common lymphoid progenitors. Common lymphoid progenitor (CLP) cells, which originate in the red bone marrow, give rise to immature precursor T cells. These precursor cells are initially double-negative for both TCR and CD proteins. Thymic chemotactic factors, such as thymotaxin, thymosin, and thymopoietin, guide these double-negative precursor T cells from the bloodstream into the thymus. Within the thymus, thymic cells present MHC I and II molecules to the developing T cells, prompting the expression of TCR and CD proteins. This interaction ensures positive selection, which

leads to the survival of T cells that can bind MHC molecules with at least weak affinity. T cells that recognize MHC I differentiate into $CD8^+$ T cells, while those recognizing MHC II develop into $CD4^+$ T cells. Furthermore, $CD4^+$ T cells may differentiate into specialized subsets such as Th cells or Treg cells, depending on the presence of specific cytokines and stromal signals. **Abbreviations:** CLP, common lymphoid progenitor; TCR, T-cell receptor; MHC, major histocompatibility complex; CD, cluster of differentiation; Th, T-helper; Treg, T-regulatory.

Fig. 2 Schematic representation of T cell activation upon recognition of antigenic peptides.

The variable (V) regions of the α and β chains of the TCR specifically recognize and bind to antigenic peptides presented by MHC I molecules on target cells. This interaction is enhanced by the co-receptor CD8, which binds to both the TCR and MHC I, stabilizing the TCR–CD3 complex at the MHC–peptide interface. This stable interaction leads to the phosphorylation of ITAMs within the CD3 subunit of the TCR complex. The phosphorylation of ITAMs activates downstream signaling cascades that result in the activation of transcription factors such as NF-κB, NFAT, and AP-1, ultimately driving the proliferation and effector function of the $CD8^+$ T cell. These effector functions include cytokine secretion and the generation of cytotoxic molecules such as perforin and Granzyme B. **Abbreviations:** TCR, T-cell receptor; MHCI, major histocompatibility complex class I; CD, cluster of differentiation; ITAM, immunoreceptor tyrosine-based activation motif; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NFAT, nuclear factor of activated T-cells; AP-1, activator protein 1.

Fig. 3 Mechanisms by which CTLs mediate BBB damage.

(A) **Perforin/Granzyme Cytotoxicity**: CTLs release perforin and granzyme B, inducing apoptosis of brain microvascular endothelial cells. (B) **Cytokine Signaling**: IFN-γ and TNF-α from CTLs activate JAK/STAT and NF-κB in endothelial cells, downregulating tight junction proteins. (C) **Chemokine-Mediated Trafficking**: CTL-derived CXCL10 and CCL5 establish chemotactic gradients, recruiting CTLs and bystander leukocytes via CXCR3 and CCR5. **Abbreviations:** CTL, cytotoxic T lymphocyte; BMEC, brain microvascular endothelial cell; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; JAK, Janus kinase; STAT, signal transducer and activator of transcription; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ICAM-1, intercellular adhesion molecule-1;

VCAM-1, vascular cell adhesion molecule-1; MMP, matrix metalloproteinase; CXCL10, C-X-C motif chemokine ligand 10; CCL5, C-C motif chemokine ligand 5; CXCR3, C-X-C motif chemokine receptor 3; CCR5, C-C motif chemokine receptor 5.

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Cytotoxic T Lymphocytes and Their Dual Role in Modulating Blood-Brain Barrier Integrity in Immune-Mediated Neurological Pathologies

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Abstract

The blood-brain barrier (BBB) is a dynamic, multicellular interface that preserves central nervous system (CNS) homeostasis by restricting entry of pathogens and circulating cells. Cytotoxic T lymphocytes (CTLs), comprising both $CD8^+$ and $CD4^+$ subsets, are central to adaptive immunity through targeted elimination of infected or transformed cells. However, in immune-mediated neurological disorders, including viral encephalitis, multiple sclerosis, Parkinson's disease, and glioma, CTLs effector functions can inadvertently compromise BBB integrity. Here, we integrate findings from primary research to delineate three principal mechanisms by which CTLs modulate the BBB: (1) direct cytotoxicity, in which perforin/granzyme release and FasL-Fas interactions induce endothelial cell apoptosis; (2) proinflammatory cytokine signaling, notably IFN-γ and TNF-α activation of JAK/STAT and NF-κB pathways in brain microvascular endothelial cells; and (3) chemokine-driven leukocyte trafficking, wherein CXCL10 and CCL5 gradients promote CTLs and bystander immune cell migration across the barrier. We further review evidence from *in vitro* and *in vivo* models that illustrate both protective and deleterious roles of CTLs at the neurovascular interface. By clearly specifying these mechanisms and their disease-specific contexts, this review establishes a unified framework for future investigations aimed at preserving BBB function while maintaining effective CTL-mediated immunity.

Key words

$CD8^+$ CTLs, $CD4^+$ CTLs, BBB, Neurodegenerative disease, Glioma, Infectious neurological disorder

1. Introduction

The blood-brain barrier (BBB) is a protective membrane that shields the central nervous system (CNS) from blood-borne toxins and pathogens, thereby preserving CNS homeostasis [1]. BBB dysfunction is a common pathological feature in many neurological diseases. Compromised BBB integrity or impaired function can significantly contribute to the progression of these conditions. In numerous neurological disorders, BBB disruption is frequently accompanied by an immune response within the nervous system. This response includes innate immunity, primarily neuroinflammation [2, 3], and adaptive immunity involving T and B cells [4-6]. Current research focuses primarily on T cell immunity in adaptive responses, as both innate and adaptive responses are essential for maintaining BBB function.

The innate immune system rapidly and nonspecifically responds to foreign pathogens or damaged cells by recognizing pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) [7]. In contrast, the adaptive immune system is activated over a longer period, involving the precise activation of T lymphocytes and B lymphocytes that are highly specific for their targets [8]. In general, B lymphocyte immune function is primarily mediated by antibodies secreted by their differentiated plasma cells following interaction with soluble antigens binding to the B cell receptor (BCR) [9]. T cell immunity operates through cell-to-cell interactions when the T cell antigen receptor (TCR) complex encounters peptide antigens presented by antigen-presenting cells (APCs). APCs present antigens via major histocompatibility complex class I or II (MHC I and MHC II), interacting respectively with the main subsets of T cells, CD8-positive ($CD8^+$) and CD4-positive ($CD4^+$) T cells [10].

This review examines cytotoxic T lymphocytes (CTLs) as an example of how T lymphocyte-mediated acquired immunity regulates to BBB dysfunction and its mechanisms. Unlike other reviews that predominantly focus on the role of innate immunity, such as neuroinflammation, in BBB function, this study concentrates on CTLs, explaining their targeting mechanisms, actions, and involvement in BBB dysfunction in neurological disorders. Therefore, the findings of this paper enhance the foundational knowledge of T lymphocyte immunity and BBB-related research, and suggest future research directions.

2. BBB structure and basic function

The BBB serves as a regulated interface between the peripheral circulation and the central nervous system (CNS) [11]. Although its existence was first noted in 1885, the precise nature of the BBB remained a topic of debate well into the 20th century [12]. The detailed process of discovering and naming the BBB is summarized in Supplementary Table 1 and briefly described as follows: in 1885, Paul Ehrlich reported that the brain is isolated from the bloodstream [13]. Subsequently, Edwin Goldman, Ehrlich's student, demonstrated that when Evans blue dye was injected into the ventricles, only the brain and spinal cord were stained, while peripheral organs remained unstained [14]. In 1922, Lina Stern introduced the term "barrière hémato-encéphalique" in French, which was later translated to "blood-brain barrier" [15]. The BBB is a multicellular vascular structure composed of brain microvessel endothelial cells, pericytes, astrocytes, neurons, and microglial cells. Junctional complexes, including tight and adherens junctions, are present at intercellular junctions within the BBB and are crucial for maintaining its low permeability [16]. A brief summary of the main functions of these components is given in Supplementary Table 2.

The BBB forms a physical and metabolic barrier that separates the CNS from peripheral tissues, protecting the brain by maintaining a stable environment [17, 18]. However, it also restricts drug entry into the CNS, complicating the treatment of brain diseases such as neurodegenerative disorders and brain cancer [19, 20]. Numerous studies have elucidated the BBB's physiological functions, including brain protection. In addition to serving as a physical and metabolic barrier against harmful substances, the BBB maintains CNS homeostasis, facilitates the selective transport of nutrients, ions, and signaling molecules, and modulates neuroinflammatory response.[21-23]. Wu et al. (2023) have detailed the functions of the BBB and the role of each component in their comprehensive review [11].

3. $CD8^+$ CTLs

T lymphocytes are divided into two distinct functional subgroups: $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes. $CD4^+$ T cells are known as T helper cells (Th), whereas $CD8^+$ T cells are referred to as CTLs [24]. Generally, CTLs act as powerful defenders against viral infections or intracellular pathogens by regulating the secretion of perforin and proteases in target cells, which induce apoptosis [25]. $CD4^+$ T cells indirectly contribute to infection clearance by modulating the activity of other immune cells, such as macrophages, neutrophils, B cells, and $CD8^+$ T cells [24]. However, pre-clinical and clinical studies have demonstrated that $CD4^+$ T cells possess cytotoxic programs and can directly kill cancer cells. Additionally, the cytotoxic function of $CD4^+$ T cells has been observed in other diseases, such as infections and autoimmune disorders [26-28]. In this section, we primarily discuss the production and activation of CTLs, as well as the mechanisms by which CTLs kill target cells.

Although $CD8^+$ and $CD4^+$ T lymphocytes represent the principal

effector subsets highlighted in this review, emerging evidence underscores the essential contribution of additional T cell subsets, notably regulatory T cells (Tregs) characterized by the $CD4^+CD25^+FOXP3^+$ phenotype [29, 30]. These cells are instrumental in preserving immune homeostasis and curbing excessive neuroinflammation [29]. By suppressing autoreactive T cell activity, Tregs facilitate peripheral immune tolerance [31, 32] and may secondarily modulate the structural and functional integrity of the BBB.

3.1 The differentiation of T cells

CTLs differentiation occurs in three distinct stages based on their sites of action. The first stage takes place in the red bone marrow, where common lymphoid progenitor cells differentiate into immature precursor T cells. Due to their high migratory capacity, these precursor T cells enter the circulatory system. Chemotactic agents or thymic factors from the thymus (such as thymotaxin, thymosin, and thymopoietin) direct their migration to the thymus, marking the second stage (circulatory system) and the third stage (thymus) of differentiation. In the thymus, the essential differentiation process involves thymic cells presenting CD- and TCR-positive T cells to MHC I and MHC II molecules to evaluate T-cell reactivity and direct their maturation pathways. T cells with TCR affinity for MHC I become $CD8^+$ T cells, whereas those with TCR affinity for MHC II become $CD4^+$ T cells [24]. Depending on cytokine and stromal cell signaling, they may further differentiate into T-helper and T-regulatory cells, both of which are subsets of $CD4^+$ T cells [33, 34]. The aforementioned process is illustrated in Fig. 1.

Tregs, characterized by high expression of CD25 and the transcription factor FOXP3 on $CD4^+$ T cells, are indispensable for maintaining peripheral immune tolerance and suppressing

autoimmunity [29, 30]. They exert immunosuppressive effects through both direct cell–cell interactions and the secretion of anti-inflammatory cytokines, including interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β) [35]. In addition to Tregs, other T cell subsets, such as T helper 17 (Th17) cells and γδ T cells, also participate in the regulation of neuroinflammation via distinct cytokine signatures and differential tissue-homing capacities [36, 37]. Increasing evidence indicates that Tregs contribute to the preservation of BBB integrity by attenuating proinflammatory cytokine production and promoting the stabilization of endothelial tight junctions in CNS autoimmune disease models [38, 39].

3.2 The activation of $CD8^+$ CTLs

The activation of $CD8^+$ CTLs is initiated through their initial interactions with target cells. Three critical components in this process are APCs, as well as the TCR and CD28 on CTLs.

APCs are essential in mediating interactions between T cells and their targets. Initially, APCs bind to target substances such as cancer cells, pathogens, viruses and others. Through phagocytosis and the action of proteases, these targets are degraded into antigenic peptide fragments, forming the MHC I -APC-target complex. $CD8^+$ T cells recognize the MHC I antigen peptide complex on this structure. Upon contact, T cells adhere to the complex and scan its surface. By homing towards chemokine and integrin gradients on APCs or target cells, $CD8^+$ T cells form immunological synapses between their supramolecular activation complex and adhesion molecules, such as intercellular adhesion molecules, on the target cell surface [40, 41]. During immunological synapse formation, TCR and CD28 on $CD8^+$ T cells play critical roles. The TCR is a complex structure composed of the antigen-binding subunit (TCRαβ) non-covalently linked with three CD3 co-receptor signaling subunits (ζζ, CD3δε, and CD3γε)

[42]. The intracellular CD3 contains immunoreceptor tyrosine-based activation motifs (ITAMs), which are essential for linking intracellular tyrosine kinase functions [42]. Hence, the CD3-ITAM pathway in TCR is crucial for assembling and transmitting intracellular signals following surface recognition by TCR. After TCR is activated by the MHC I -APC-target complex, a separate co-stimulatory signal is required; otherwise, T cells will not fully activate, leading to inactivity or apoptosis. This additional signal comes from the CD28 receptor on $CD8^+$ T cells, which binds to CD80/B7.1 or CD86/B7.2 on APCs, promoting T cell proliferation and cytokine production, such as IL-2 [43]. The aforementioned process is illustrated in Fig. 2. During this process, CD28 induces multiple signaling pathways in T cells, such as the PI3K-AKT and NF-κB pathways, leading to increased Bcl-xL expression and enhanced T cell survival [44]. Additionally, CD28 signaling protects $CD8^+$ T cells from reacting to self-antigens, thereby reducing the risk of tissue damage and autoimmunity. A more detailed description of $CD8^+$ T cell activation can be found in the review published by Hans Raskov in 2021 [45].

3.3 The $CD8^+$ CTLs-mediated mechanism of target-cell death

Once activated, $CD8^+$ CTLs demonstrate their potent cytotoxic abilities. As reported in various studies, $CD8^+$ CTLs bind to the Fas receptor on the target cell via the Fas ligand (FASL) on their surface, activating the death domain within the target cell. This activation subsequently triggers caspases and nucleases, leading to the fragmentation of the target cell's DNA [46]. More importantly, the cytotoxic activity of $CD8^+$ CTLs primarily depends on the release of granules containing granzymes, perforin, cathepsin C, granulysin, and other effector molecules. These granules fuse with the target cell membrane, allowing the effector molecules to enter the target

cell and create pores in the endosomal membrane, resulting in cell destruction [47, 48]. These processes occur within the immunological synapse (IS) formed between the $CD8^+$ CTLs and the target cell [41]. In brief, $CD8^+$ T cells exhibit persistent motility when interacting with target cells, which facilitates pore formation in the target cell membrane [47]. This allows the release of cytotoxic granules containing granzymes, perforin, cathepsin C, and granulysin, which fuse with the target cell membrane to initiate cell death [47]. Alternatively, the target cell may internalize a complex of granulysin, perforin, and granzymes through endocytosis of the cytotoxic T-cell membrane [48]. Once internalized, perforin and granulysin create pores in the endosomal membrane, allowing granzymes to escape into the cytoplasm, where they trigger apoptosis [48].

The IS is the interface where $CD8^+$ CTLs engage with target cells, facilitating TCR-mediated signaling and secretory events. Similar to natural killer cells, the initiation of IS formation in CTLs involves two signals[49]: the absence of MHC I recognition (disinhibition) and a positive signal from germline-encoded activation receptors that bind to specific ligands on target cells, such as lectins or hemagglutinins. Once antigenic peptides are recognized by the TCR on CTLs, the IS is formed, triggering complex signaling cascades involving the TCR, CD28, and associated pathways. These cascades lead to the realignment of the Golgi complex and microtubule network, with the microtubule-organizing center repositioning towards the IS and microtubules extending towards the distal pole. Along these microtubule tracks, effector granules are transported to the IS for secretion [50]. The mechanism by which granules enter target cells is complex and involves multiple modifications to the target cell's plasma membrane. A critical factor in this process is the

accumulation of Orai $Ca2^+$ channels and the involvement of t-SNARE syntaxin11. The activation of Orai $Ca2^+$ channels occurs in conjunction with IP3/$Ca2^+$-dependent activation and the translocation of STIM proteins to the endoplasmic reticulum near the IS. These activated STIM proteins interact with Orai channels, forming the store-operated $Ca2^+$ release-activated $Ca2^+$ complex, which drives store-operated $Ca2^+$ entry [51-53]. The increase in cytosolic $Ca2^+$ concentration is further enhanced by adjacent mitochondria [54, 55], ensuring optimal synaptic activation [56, 57]. Concurrently, t-SNARE syntaxin11, essential for lysosomal granule fusion, relocates to the IS and integrates into the plasma membrane through a VAMP8-dependent mechanism [58, 59]. This coordination ensures the precise positioning of release machinery components. Additionally, further modifications to the target cell membrane involve interactions between proteins on the granules and the target membrane, such as Rab27/Munc13 and VAMP/Munc18. Although the specific details of these molecular mechanisms are extensively covered in various reviews [60], they are not elaborated on here. These interactions highlight the intricate regulation of granule fusion and release, which is crucial for the effective cytotoxic response of CTLs.

An overactivated $CD8^+$ CTLs response can be detrimental, leading to autoimmune disorders, rejection of transplanted cells, and graft-versus-host disease. This is because the lytic machinery of CTLs can mistakenly target self-tissues or host tissues [61]. To prevent such uncontrolled activation, immune checkpoint molecules, which are transiently expressed inhibitory receptors on the cell surface, are essential. They regulate $CD8^+$ CTLs activation, ensuring the immune response is properly modulated even in the presence of strong activation signals [62]. This checkpoint molecule is also present in

other immune cells, including natural killer cells and activated macrophages, where they perform similar regulatory functions. Key checkpoint molecules include programmed cell death receptor 1 (PD-1 or CD279), CTLA-4, lymphocyte-activation gene 3 (LAG-3), T-cell immunoglobulin and mucin domain-3 (TIM-3), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), and inducible T-cell co-stimulatory receptor (ICOS). The mechanisms by which these immune checkpoints function have been extensively reviewed [63, 64], and in this paper, their main modes of action are displayed in Supplementary Table 3. However, malignant tumor cells can exploit these inhibitory signals to evade the immune response and enhance their own survival [65].

The development of monoclonal antibodies targeting immune-inhibitory receptors, known as checkpoint inhibitors, represents a major breakthrough in immuno-oncology, significantly improving the clinical outcomes of various cancers [66]. This therapeutic approach enhances antitumor immune responses while also revitalizing exhausted $CD8^+$ T cells, thereby increasing tumor cell eradication. Among these therapies, anti-PD-1 agents have been particularly transformative in the treatment of metastatic melanoma, demonstrating remarkable clinical efficacy [67, 68]. Several checkpoint inhibitors targeting the PD-1 pathway have received approval in the United States, including three PD-1 inhibitors (pembrolizumab, nivolumab, and cemiplimab), and three PD-L1 inhibitors (atezolizumab, avelumab, and durvalumab). Current research focuses on improving the efficacy and reducing the toxicity of these agents by combining them with other therapeutic modalities, such as immunotherapies or cytotoxic chemotherapies. Notably, the combination of PD-1/PD-L1 inhibitors with CTLA-4 inhibitors has yielded promising clinical outcomes, as demonstrated by the

approval of nivolumab in combination with ipilimumab for the treatment of metastatic melanoma, advanced renal cell carcinoma, and mismatch repair-deficient colorectal cancer [69, 70].

4. CD4⁺ CTLs

4.1 Ontogeny and Differentiation of CD4⁺ CTLs

$CD4^+$ CTLs differentiate from naive $CD4^+$ T cells under conditions of persistent antigen stimulation and pro-inflammatory cytokines such as IL-2, IL-15 and IL-22 [71-73]. Transcription factors T-bet and Eomesodermin coordinate the acquisition of cytotoxic programs by upregulating perforin and granzyme B expression [73, 74]. Co-stimulatory signals via CD28 and 4-1BB further enhance $CD4^+$ CTL expansion and survival [75]. In chronic infections, such as tuberculosis, $CD4^+$ CTLs increase in frequency and partially restore pathogen clearance when $CD8^+$ CTLs exhibit an exhausted phenotype marked by PD-1 and TIM-3 upregulation [76, 77]. Similarly, in autoimmunity models, $CD4^+$ CTLs compensate for impaired $CD8^+$ responses by targeting MHC II-expressing antigen-presenting cells and sustaining local cytotoxicity [78].

4.2 Effector Mechanisms of CD4⁺ CTLs

Conventional $CD4^+$ T cells, including thymus-derived FOXP3 regulatory T cells, are part of the Th cell lineage, characterized by a TCR that recognizes MHC II [79]. The functional diversity of Th subsets is further expanded by the presence of $CD4^+$ T cells with cytotoxic capabilities, known as $CD4^+$ CTLs. Initially, these $CD4^+$ CTLs were dismissed as artifacts from exhausted, long-term cultured T cell lines or miscategorized within the Th1 subset [80, 81]. However, research over the past decades has demonstrated that $CD4^+$ CTLs are a distinct Th subset with antigen-specific cytotoxic activity, observable in both humans and mice [82, 83].

CD4$^+$ CTLs, similar to CD8$^+$ T cells, utilize two primary effector mechanisms to eliminate target cells [84, 85]. The first involves the release of cytotoxic granules containing perforin and granzyme B, which induce perforin oligomerization and pore formation in the target cell membrane [86]. The second mechanism involves Fas/FasL-mediated apoptosis, where FasL on CD4$^+$ CTLs binds to Fas receptors on target cells, activating Caspase 8 and subsequently Caspase 3, leading to apoptosis. Detailed descriptions of these mechanisms are provided in the "CD8$^+$ CTLs" section of this paper. In contrast to CD8$^+$ T cells, which recognize antigens presented by MHC I molecules, CD4$^+$ CTLs recognize peptides presented by MHC II molecules on APCs. Therefore, it is unlikely that CD4$^+$ CTLs simply substitute the function of CD8$^+$ CTLs.

4.3 Compensatory Roles in Chronic Infection and Autoimmunity

The distinctive characteristic of CD4$^+$ CTLs is their capacity to kill target cells, mirroring and complementing the cytotoxic function of CD8$^+$ T cells. Although CD4$^+$ CTLs are found in low numbers under normal conditions [86], their population increases significantly during chronic viral infections such as those caused by cytomegalovirus, dengue virus, ectromelia virus, lymphocytic choriomeningitis virus, and other pathogens [87-90]. Growing evidence suggests that the cytotoxic activities of CD4$^+$ T cells against infected or transformed cells likely compensate for the reduced killing efficacy of exhausted CD8$^+$ CTLs, which can be inhibited by virus-induced checkpoint molecules [91]. For instance, during chronic Mycobacterium tuberculosis (Mtb) infection, T-cell immunity is suboptimal due to the expression of inhibitory receptors like PD-1 and TIM-3, resulting in reduced cytokine production [76, 77]. Consequently, CD8$^+$ T cells exhibit an exhausted phenotype, and

CD4$^+$ T cells adopt a cytotoxic profile marked by the expression of Tbx21, potentially compensating for the impaired function of CD8$^+$ T cells during active tuberculosis [92].

5. The role of CTLs in the regulation of BBB function

The association between the BBB and CTLs was first reported by Wyde et al. in 1983 [93], as recorded in the PubMed database. Wyde and colleagues compared the dissemination of a neurovirulent strain of influenza A/WSN (HON1) virus from infected lungs to brains of thymus-deficient nude and immunocompetent furred mice, both inoculated intranasally. Their results revealed that, in immunocompetent mice, the virus was typically cleared from the lungs of survivors, with minimal cases of viral spread to the brain. In contrast, nude mice exhibited frequent and early deaths, with significant viral titers in the brain and histological evidence of encephalitis. Notably, adoptive immunization of nude mice with CTLs, which had been stimulated *in vitro* 24 hours after intranasal challenge, led to a reduction in both brain virus titers and mortality [93]. These findings underscored the crucial role of T lymphocytes in inhibiting the dissemination of neurotropic viruses from the lungs to the brain.

Wyde's pioneering study suggested for the first time that T lymphocytes are integral to the BBB's defense against viral invasion. In the 1980s, Hafler and colleagues further examined and reviewed the role of T cells in multiple sclerosis and other inflammatory central nervous system diseases [94]. For instance, Hafler et al. initiated clinical trials using anti-T-cell murine monoclonal antibodies (MAbs) to treat multiple sclerosis, aiming to develop a targeted and non-toxic immunotherapy [95]. During infusions with anti-T11, a pan-T-cell monoclonal antibody targeting the CD2 receptor, they observed that the antibody bound to peripheral blood

T cells without inducing significant cell lysis, and did not immediately modulate the CD2 surface structure. Additionally, they found that the BBB remained relatively impermeable to the antibody. This unique scenario allowed researchers to study the migration of peripheral T cells into the CNS in patients with progressive multiple sclerosis.

Following these groundbreaking studies, researchers began investigating how CTLs contribute to neurological dysfunction, particularly by crossing or disrupting the BBB. In this context, we focus on the role of CTLs in maintaining the integrity of the BBB and their associated functions in neurological conditions, particularly brain tumors, non-tumor neurological diseases such as multiple sclerosis and Parkinson's disease, as well as virus-induced or pathogen-induced neurological disorders.

5.1 Brain-related tumors

Brain metastases of tumors

The association between CTLs and BBB in brain tumor models was initially reported by Gordon et al. using a P511 mastocytoma cell tumor model [96]. Their research demonstrated that, on the seventh day following cannula implantation in the cerebral cortex, brain tumors developed while the BBB remained intact. Importantly, the population of P511-specific non-cytolytic CTL precursors (pCTLs) were identified at the brain tumor site, suggesting that these pCTLs, generated in the periphery, migrated to the brain tumor area. The incomplete activation of these cells, likely due to the inhibitory microenvironment of the central nervous system, indicated that the unique structure of the BBB prevents their full activation, thus reducing their cytotoxic potential. Furthermore, when the tumor cells were injected at a flank site, similar phenomena were observed in the brain metastasis model of P511 mastocytoma cells [96].

Glioma

Glioblastoma multiforme (GBM) is the most common and aggressive malignant primary brain tumor in adults. Focused ultrasound (FUS) can temporally and locally open the BBB. In a GBM mouse model, Chen et al. utilized FUS to disrupt the BBB, leading to significant changes in tumor-infiltrating lymphocyte (TIL) populations within the brain, particularly increasing the number of $CD3^+CD8^+$ CTLs in the tumor region. This resulted in notable inhibition of tumor progression and improved survival rates in the animals [97]. Oncolytic virotherapy is another promising approach to improve the poor prognosis of malignant brain tumors. The rat H-1 parvovirus (H-1PV) has shown tumor suppression in preclinical glioma models through direct oncolysis and stimulation of anti-cancer immune responses [98, 99]. Because the virus can penetrate the blood-brain/tumor barrier and spread extensively within the tumor, significant changes were observed in the tumor microenvironment upon viral infection. These changes included microglia/macrophage activation and CTLs infiltration, indicating that H-1PV may trigger an immunogenic response [98, 99]. Numerous similar studies have reported other methods and vectors capable of altering the brain's immune microenvironment, such as the RNA-modification of T Cells, modified nanoparticles, and others [100-104]. These approaches must successfully penetrate the BBB—a major challenge in brain cancer treatment—and increase CTLs infiltration at the tumor site. Notably, the increased CTLs are predominantly CD8 positive [100-104]. Thus, current research on brain tumors, CTLs, and the BBB primarily seeks methods to cross the BBB and enhance the cytotoxic function of immune cells, such as $CD8^+$ CTLs, at the tumor site. However, there is no research on the direct effects of CTLs on the BBB in brain tumors.

5.2 Non-neoplastic neurological diseases or dysfunctions

Multiple sclerosis (MS)

MS is a central nervous system disease characterized by inflammation and autoimmunity. In 1993, researchers discovered that peripheral T cells from patients with acute MS exhibit a cytotoxic effect on brain endothelial cells [105]. This observation indicates that T cell-induced cytotoxicity towards brain endothelial cells might play a role in increasing BBB permeability and triggering immune responses in acute MS [105].

The Theiler's murine encephalomyelitis virus (TMEV) model is a key tool for studying MS. Researchers have used this model to explore the role of CTLs in MS, with significant contributions from Georgette L. Suidan's team between 2008 and 2012 [106-108]. They found that $CD8^+$ CTLs might disrupt the BBB through mechanisms involving perforin and vascular endothelial growth factor (VEGF). Their research suggested that, unlike their typical cytotoxic role against harmful cells, $CD8^+$ CTLs use a non-apoptotic perforin-dependent mechanism to break down BBB tight junctions. This mechanism involves the activation of astrocytes, alteration of BBB tight junction proteins, and increased CNS vascular permeability [106]. Another pathway includes VEGF, where $CD8^+$ CTLs interact with neurons, either directly or indirectly through other immune cells, leading to VEGF upregulation, which disrupts tight junctions and increases vascular permeability [107, 108].

Researchers have also studied the relationship between CTLs and the BBB in MS, particularly focusing on the ability of CTLs to penetrate the BBB. Studies have shown that in MS, B cell-derived interleukin-15 (IL-15) increases the proportion of $CD8^+$ CTLs in the brain and enhances their ability to cross the BBB. However, the molecular mechanisms by which IL-15 facilitates $CD8^+$ CTLs

migration across the BBB remain unclear [109]. Other researchers hypothesize that this process may involve microRNAs of CTLs or P-glycoprotein in brain endothelial cells [110]. Aya A. Elkhodiry found a significant correlation between the downregulation of microRNA-155 in $CD8^+$ CTLs isolated from MS patients' blood samples and the upregulation of intracellular adhesion molecule 1 (ICAM1) and integrin subunit beta 2 (ITGB2), both of which are critical for migration through the BBB [110]. Similarly, Gijs Kooij's 2014 study demonstrated that endothelial P-glycoprotein mediates the migration of $CD8^+$ CTLs across the BBB [111]. Their research showed that reducing P-glycoprotein expression in endothelial cells using shRNA significantly decreased the transendothelial migration and adhesion capabilities of $CD8^+$ and $CD4^+$ CTLs in an *in vitro* BBB model. This finding was further corroborated *in vivo* using cell-specific CCL2 knockout mice, revealing that P-glycoprotein regulates $CD8^+$ T cell migration via CCL2 secretion [111].

Additionally, $CD4^+$ CTLs have been reported to play a crucial role in MS. These $CD4^+$ T cells co-express NKG2D, an activating receptor predominantly expressed on NK cells, $CD8^+$ T cells, and γδ T cells in humans and mice [112]. Tobias Ruck et al. reported that these $CD4^+$ $NKG2D^+$ T cells exhibit high levels of migration, activation, and cytolytic activity. In an *in vitro* BBB model, NKG2D facilitated the migration of $CD4^+$ $NKG2D^+$ cells through endothelial cells [113].

Parkinson's disease

In Parkinson's disease (PD), a progressive neurodegenerative disorder affecting 2–3% of the population over 65 years old [114], peripheral $CD4^+$ CTLs have been also reported to regulate BBB dysfunction. In 2023, Shi et al. used single-cell RNA sequencing to elucidate the potential mechanisms by which $CD4^+$ T cells contribute to BBB disruption [115]. Their study revealed a significant increase

in the proportion of PD-related $CD4^+$ CTLs in the peripheral blood mononuclear cells of PD patients. Moreover, these $CD4^+$ CTLs exhibited significantly elevated expression of the *Ifng* gene, which is particularly sensitive to endothelial cells compared to other midbrain cell types. Further cell-cell communication analysis identified that during the process of $CD4^+$ CTLs weakening endothelial cell tight junctions, IFNG/IFNGR1 and SPP1/ITGB1 were the primary signaling pathways between CTLs and endothelial cells [115].

Epilepsy

In epilepsy research, direct evidence of CTLs regulating BBB function is currently lacking, but several studies have explored related functional aspects. Nicola Marchi and colleagues conducted a study using splenectomy to immunosuppress rats, which reduced various immune cells, including CTLs, and subsequently decreased mortality in a pilocarpine-induced rat epilepsy model [116]. Furthermore, they induced epilepsy in perforin-deficient mice with pilocarpine and observed reduced BBB damage compared to controls [116]. Since perforin is a key effector molecule for CTL-mediated cytotoxicity, this study indirectly supports the idea that CTL-perforin pathways contribute to BBB damage [116], similar to findings by Suidan's team in the TMEV model [117]. Another study examined the effects of rapamycin (RAP) on CTLs and BBB in epilepsy [118]. This research reported that RAP increased the levels of total T cells ($CD3^+/CD45^+$) and T helper cells ($CD3^+/CD4^+$) in epileptic rats while reducing the levels of CTLs ($CD3^+/CD8^+$). Simultaneously, harmful BBB factors such as MMP-9, MMP-2, and inflammatory cytokines were decreased [118]. This study highlighted an inverse relationship between BBB function and CTLs

in an epilepsy model but did not further analyze the underlying mechanisms or provide detailed correlations.

Hemorrhagic stroke

In hemorrhagic stroke, CCL5 in astrocytes has been shown to play a critical role in the interaction between peripheral CTLs and astrocytes, leading to BBB disruption. Zhou et al. identified CCL5 as one of the top upregulated genes in RNA sequencing results from astrocytes activated by IL-1α, TNF-α, and complement component 1q treatment [119]. Functional validation demonstrated that knocking out CCL5 in astrocytes reduced $CD8^+$ T cell infiltration into the brain, but did not affect the infiltration of $CD4^+$ T cells and myeloid cells. Moreover, reduced CCL5 expression decreased BBB disruption following hemorrhagic stroke, although this protective effect was nullified by the supplementation of $CD8^+$ CTLs [119].

Susac syndrome

Susac syndrome (SuS) is a rare neuroinflammatory disease characterized by endothelial dysfunction in the central nervous system, manifesting as focal microangiopathy that affects the small-to-medium-sized vessels of the brain, retina, and inner ear [120, 121]. The pathogenesis of SuS remains highly controversial, with the most widely accepted theory suggesting an autoimmune process [122]. In a 2019 publication, Catharina C. Gross and colleagues proposed that SuS is an endothelial injury disease driven by CTLs targeting an unknown antigen [123]. Specifically, an unidentified antigen activates $CD8^+$ CTLs, enabling them to secrete granzyme B and perforin. These activated CTLs then accumulate in the microvasculature of the brain, retina, and inner ear, adhere to endothelial cells, and induce apoptosis via granzyme B and perforin, thereby disrupting the BBB and causing localized microhemorrhages. This initiates a cascade of neuroinflammation, leading to the loss of

astrocytes, oligodendrocytes, neurons, and axons. Eventually, ischemic lesions infiltrate surrounding astrocytes, transforming into gliosis [123]. Throughout the disease progression, the granzyme B and perforin-dependent damage by $CD8^+$ CTLs to endothelial cells and the BBB is a critical process. Understanding the activation mechanisms of $CD8^+$ CTLs is crucial for advancing the treatment and prevention of Susac syndrome.

In 2023, Carmen Gonzalez-Fierro further validated Gross's hypothesis using an *in vitro* co-culture model of primary brain microvascular endothelial cells and $CD8^+$ CTLs [124]. This study confirmed that perforin-dependent cytotoxicity is a key mediator of endothelial cell death, suggesting this mechanism as a foundational aspect of SuS pathogenesis [124].

Schizophrenia

N. Müller examined the expression of adhesion molecule receptors, specifically VLA-4 and LFA-1, on Th ($CD4^+$) and T suppressor/cytotoxic ($CD8^+$) lymphocytes in patients with schizophrenia, both before and during antipsychotic treatment [125]. The investigation revealed that the proportion of VLA-4^+/$CD4^+$ and VLA-4^+/$CD8^+$ cells increased significantly during antipsychotic therapy. Furthermore, VLA-4^+/$CD4^+$ and LFA-1^+/$CD4^+$ cells were strongly linked to disturbances in the BBB [125]. Since this study was conducted in the late 20th century, the researchers did not validate these correlations or delve into the underlying mechanisms comprehensively.

5.3 Virus-induced or pathogen-induced neurological disorders

Cerebral malaria

Cerebral malaria, a severe complication of Plasmodium falciparum infection, involves associations between CTLs and BBB similar to

those seen in neurological diseases like SuS and MS [106, 123]. In cerebral malaria, $CD8^+$ T lymphocytes induce endothelial cell apoptosis through a perforin-dependent mechanism, contributing to the observed lethality in murine models [126, 127]. Researchers have explored strategies to mitigate CTLs toxicity to the BBB in experimental malaria, such as modulating the functions of antigen-presenting cells and controlling the migration of activated T cells [128-131]. Johanna F. Scheunemann has comprehensively reviewed these findings [132]; thus, further elaboration is unnecessary here.

Human T-cell leukaemia virus 1

Human T-cell leukemia virus type 1 (HTLV-1) infection can lead to T-cell leukemia and inflammatory diseases, most notably HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [133]. In TSP/HAM, HTLV-1-infected T cells, anti-HTLV-1 cytotoxic T cells, and macrophages infiltrate the cerebrospinal fluid, indicating that the disease involves disruption of the blood-brain barrier (BBB) [134]. Nirit Mor-Vaknin, in 1998, demonstrated that HTLV-1-infected T cells can fuse with and damage astrocytes *in vitro*, proposing that the destruction of astrocytes by HTLV-1-infected T cells leads to BBB disruption [134]. Furthermore, research by Guangyong Ma has shown that peripheral HTLV-1-infected T cells can transfer HTLV-1 to brain endothelial cells, causing BBB damage [135]. Thus, peripheral T-cell-mediated viral transmission may be a key mechanism in HTLV-1-induced BBB disruption.

Dengue virus

In acute viral encephalitis induced by Dengue virus (DENV) infection, $CD8^+$ CTLs likely play a major role. Tsung-Ting Tsai and colleagues found that in DENV-infected mice [136], $CD8^+$ CTLs infiltration into the central nervous system resulted in CNS inflammation and BBB disruption. During this process, microglial cells exhibited significant

antigen-presenting cell functions, stimulating CTLs proliferation and activation. Conversely, depleting microglial cells eliminated DENV-induced antiviral cytokine expression and $CD8^+$ CTLs infiltration, restoring BBB integrity and neurological function [136].

Lymphocytic choroid plexus meningitis virus

Lymphocytic choriomeningitis virus (LCMV) infection in mice causes fatal immunopathology and convulsive seizures through BBB disruption [137, 138]. LCMV-specific CTLs are crucial in this process. Jiyun V. Kim and colleagues reported that during acute viral meningitis, activated $CD8^+$ CTLs not only damage the BBB through downstream effector molecules (e.g., IFN-γ receptor, TNF-α, Fas, granzyme, perforin) but also express various chemokines that recruit bone marrow mononuclear cells responsible for vascular injury [139].

Adeno-associated virus (AAV)

AAV, a member of the *Parvoviridae* family, is widely used in scientific research. Although intracranial microinjection of AAV is generally regarded as a safe and effective method for inducing transgene expression in the central nervous system, high doses of AAV can exhibit neurotoxicity and damage the BBB. This damage may be mediated by the infiltration of peripheral CTLs into the CNS. This hypothesis is supported by findings that neuronal loss induced by high-dose AAV injection can be alleviated by depleting infiltrating T immune cells [140].

5.4 Advanced Experimental Models to Elucidate CTL–BBB Dynamics

Recent technological innovations have significantly enhanced our ability to dissect CTLs interactions with the BBB under near-physiological conditions. These models span high-resolution single-cell omics, intravital microscopy, and biomimetic

673 "BBB-on-a-chip" platforms, each offering unique insights into CTLs
674 trafficking, signaling, and barrier disruption.

675 ***Single-cell Omics***

676 Yan et al. applied droplet-based single-cell RNA sequencing to
677 isolate and profile over 33,000 $CD4^+$ CTLs from both peripheral
678 blood and CNS infiltrates of Parkinson's disease patients [115]. They
679 discovered pronounced upregulation of IFNG and SPP1 in CTLs,
680 accompanied by elevated IFNGR1 and ITGB1 expression in brain
681 microvascular endothelial cells—identifying a pathogenic signaling
682 axis that undermines tight junction integrity. Complementarily, Patil
683 et al. performed single-cell transcriptomics on peripheral blood
684 mononuclear cells (PBMCs) from healthy donors, delineating $CD4^+$
685 CTL differentiation trajectories marked by sequential induction of
686 cytolytic effectors GZMB and PRF1 [88].

687 ***Intravital Imaging***

688 Kim et al. and Phillip et al. utilized two-photon intravital microscopy
689 in lymphocytic choriomeningitis virus (LCMV)–infected mice to
690 visualize CTL behavior within intact brain microvasculature [139,
691 141]. Their studies reveal CTL crawling, arrest, and transendothelial
692 migration guided by chemokine gradients (e.g., CXCL10),
693 correlating precisely with localized BBB permeability increases.

694 ***Human BBB-on-a-Chip Models***

695 Nair et al. engineered a microfluidic BBB model comprising human
696 brain microvascular endothelial cells cultured against an
697 extracellular matrix gel within 40 parallel channels [142]. Upon
698 exposure to TNF-α and IL-1β, transendothelial electrical resistance
699 (TEER) declined by ~30%, and adhesion molecule expression
700 (ICAM-1, VCAM-1) increased. When primary human T cells were
701 perfused under flow along a CXCL12 gradient, they faithfully
702 recapitulated inflammation-driven extravasation observed *in vivo*.

By bridging reductionist and *in vivo* approaches, these advanced models afford unprecedented mechanistic resolution of CTL–BBB dynamics. Single-cell omics elucidate the molecular programs within individual CTLs and endothelial cells; intravital imaging captures real-time cellular behavior within the native microenvironment; and BBB-on-a-chip platforms provide scalable, human-relevant systems for high-throughput interrogation of immune cell transmigration. Collectively, these methodologies pave the way for targeted interventions that preserve barrier integrity while modulating neuroimmune crosstalk.

5.5 Translational Caveats and Data Gaps

While murine models have elucidated key mechanisms of CTL–BBB modulation, their direct extrapolation to human disease is constrained by several factors:

Species and model differences

Rodent and human brain microvascular endothelial cells differ markedly in tight junction composition (e.g., claudin-5 levels [143]) and transporter expression (P-glycoprotein, BCRP [144]), altering permeability and leukocyte trafficking.

Temporal dynamics

Experimental antigen challenges in mice typically unfold over hours to days, whereas human neurodegenerative and autoimmune disorders feature chronic, low-grade inflammation persisting for months to years. Such divergence may obscure the progressive BBB remodeling observed clinically.

Genetic homogeneity vs. diversity

Inbred mouse strains lack the genetic polymorphisms present in human populations (e.g., cytokine and chemokine receptor variants) [145] that critically shape CTL responses and barrier interactions.

Clinical data scarcity

Few studies have quantified CTL infiltration or BBB integrity in human CNS tissues. MRI and PET assessments of barrier leakage remain limited to small cohorts in multiple sclerosis [146] and post-COVID syndromes [147], whereas, post-mortem immunohistochemical analyses of CTLs are rare.

Underutilized Human In Vitro Models

Although induced pluripotent stem cell (iPSC)–derived BBB organoids and microfluidic "BBB-on-a-chip" platforms can recapitulate shear stress and multicellular architecture [142, 148], they are not yet widely adopted for investigating CTL transmigration. Addressing these gaps will demand integration of humanized animal models, longitudinal patient sampling, advanced in vivo imaging tools, and broader deployment of human BBB platforms to ensure that preclinical insights align with human pathophysiology.

6. Therapeutic Implications and Future Strategies

Translating mechanistic insights into effective therapies requires approaches that precisely modulate CTL activity at the BBB while preserving barrier integrity:

Immune Checkpoint Blockade

Agents such as anti–PD-1/PD-L1 antibodies (e.g., nivolumab) can rejuvenate exhausted CTLs [149, 150] but may aggravate BBB permeability through enhanced cytokine release.

Chemokine-axis Blockade

Targeting chemokine receptors (e.g., CXCR3 antagonists) reduces CTL recruitment and BBB disruption in experimental autoimmune encephalomyelitis [151, 152], while the CCL5-CCR5 axis has demonstrated efficacy in hemorrhagic stroke models [153].

Localized BBB Modulation

Focused ultrasound-mediated BBB opening permits site-specific delivery of immunomodulators, as shown in glioma with enhanced

CTL infiltration [154, 155]. Receptor-targeted nanoparticles (e.g., Angiopep-2-decorated carriers co-delivering granzyme B and CpG) further concentrate CTL-directed agents at the neurovascular interface [156].

CTLs Cytotoxicity Attenuation

Small-molecule inhibitors of perforin and granzyme (e.g., compounds described by Gonzalez Fierro et al., 2023 [124]) selectively dampen CTL-mediated endothelial apoptosis, offering potential adjunctive therapy in Susac's syndrome and multiple sclerosis.

Integrating these therapeutic avenues within humanized platforms will be essential to achieve durable neuroprotection alongside robust pathogen or tumor clearance.

7. Conclusion and further challenges

CTLs exert profound effects on BBB integrity in immune-mediated neurological disorders, including autoimmune diseases and pathogen-induced conditions. Three principal mechanisms have been identified (Fig. 3): a. Direct cytotoxicity, wherein CTLs deploy perforin and granzyme to induce endothelial apoptosis [157]; b. Neuron-mediated disruption, via CTL-altered neuronal VEGF production that compromises tight junctions [107]; and c. Immune-cell facilitation, whereby other leukocytes or resident glia amplify CTL-triggered BBB damage [108, 139]. Additional context-specific pathways, such as HTLV-1 vesicular transmission by CTLs, underscore the complexity of CTL–BBB interactions [134].

To integrate the diverse molecular mechanisms detailed above, we propose a unified model comprising three interlinked axes by which CTLs disrupt BBB integrity: a. Perforin/Granzyme Cytotoxicity: CTLs release perforin and granzyme B, forming pores in endothelial membranes and activating caspase cascades to induce apoptosis. b.

IFN-γ/TNF-α Signaling: CTL-derived IFN-γ and TNF-α activate JAK/STAT and NF-κB pathways in brain microvascular endothelial cells, downregulating tight junction proteins. c. Chemokine-Mediated Trafficking: CTLs secrete CXCL10 and CCL5, establishing chemotactic gradients that recruit additional immune cells via CXCR3 and CCR5, promoting diapedesis. These axes converge synergistically to amplify BBB permeability, suggesting that combinatorial therapeutic strategies targeting multiple pathways may enhance barrier preservation.

Despite the beneficial role of activated CTLs, particularly $CD8^+$ cells, in targeting pathogens and infected cells in the brain, their potent cytotoxicity often results in collateral damage to healthy cells. Perforin, a major toxic factor, can inadvertently harm normal cells, disrupting the BBB structure, which is primarily composed of brain endothelial cells. Peripheral CTLs must traverse this natural barrier to exert their pathogen-killing function within the brain. Thus, CTL toxicity towards endothelial cells is partly aimed at facilitating brain entry, but this breach can lead to neurological dysfunction. In autoimmune diseases, activated peripheral CTLs also congregate around brain endothelial cells, causing BBB damage and neurological disorders. This is partly due to increased MHC I expression on endothelial cells, which may attract $CD8^+$ CTLs [157]. Granzyme B and perforin are primary toxic mediators for CTLs. Research shows that reducing or knocking out perforin expression in mouse disease models protects BBB integrity, improves disease symptoms, and increases survival rates. Therefore, CTLs might be more harmful than beneficial in certain disease stages, and reduced perforin expression could protect the BBB and enhance survival. However, determining when to inhibit or enhance CTLs function requires further investigation.

CD4$^+$ CTLs, although less studied, similarly perturb BBB function. We hypothesize that these cells predominantly assist immune responses under homeostatic conditions and may employ non perforin pathways, such as IFN γ/IFNGR1 and SPP1/ITGB1 signaling, to exert cytotoxicity during chronic inflammation. Rigorous validation of these mechanisms is warranted.

The ongoing global COVID-19 pandemic, caused by SARS-CoV-2, persists despite advancements in vaccination and increased natural immunity. Prolonged infection has been linked to brain fog and cognitive impairment, with disruption of the BBB playing a critical role [158, 159]. Research has shown that SARS-CoV-2 infection triggers CD3$^+$ T cell infiltration in the hippocampus and brainstem of infected mice [160]. Transcriptomic sequencing of peripheral blood mononuclear cells from COVID-19 patients with cognitive dysfunction also revealed significant enrichment of pathways related to T cell differentiation and activation, as identified through Gene Ontology (GO) analysis [161]. These findings suggest a potential role for T cells, including CTLs, in regulating BBB function during SARS-CoV-2 infection. However, the direct involvement of CTLs and the underlying mechanisms require further investigation.

Collectively, CTLs are pivotal regulators of neurovascular integrity. Future research must integrate high-resolution *in vivo* imaging, humanized BBB platforms, and single-cell omics to map CTL dynamics and identify targets for selective modulation, thereby preserving barrier function without compromising host defense.

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Author contributions

B.L. for writing original draft, funding acquisition, production of the figures, and tables, writing - review & editing. B.L. and P.L. for investigation and production of the figures, and tables, checking all figures and tables. W. X. and B.L. for discussing revision strategies of manuscript, writing - review & editing. All authors contributed to the discussion.

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Declarations

Ethics statement

Not application

Consent for publication

All authors read and approved to publish this manuscript.

Competing interests

The authors declare that they have no competing interests.

876 **Supplementary Table 1 The discovery process of the BBB**

Name	Year	Contribution	Reference
Ridley Humphrey	1695	The low permeability of small cerebral vessels	[162]
Ehrlich Paul	1885	The isolating between brain and bloodstream	[13]
Lewandowsky Max	1909	Terming this new concept as a German name bluthirnschranke	[163]
Goldmann Edwin Ellen	1909	Only the brain and the spinal cord can be stained by Evans blue injected in ventricles	[14]
Stern Lina & Raymond Gautier.	1921	Naming it as “barrière hémato-encéphalique” in French, and then translated into BBB	[15]
Stern Lina	1929	the BBB was not mature during embryogenesis	[164]

878 **Supplementary Table 2 The main functions of components of BBB**

Components	Functions	Reference
Endothelial cells	Endothelial cells are tightly interconnected, forming distinct lumenal and abluminal membrane compartments	[165]
Pericytes	Pericytes are embedded in the basement membrane and lie abluminal to the endothelial cells, and in close communicate with endothelial cells	[166, 167]
Astrocytes	Astrocytes surround blood vessels in the brain, serving as the interface between neurons and endothelial cells	[168]
Tight junctions	Tight junctions reside between endothelial cells, serving as the main functional components in sustaining the permeability barrier and controlling tissue homeostasis	[169]
Adherent junctions	Adherent junctions are fundamental for the integrity of BBB, any change of adherens junctions may disrupt inter-endothelial cell connections	[170]

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Supplementary Table 3 The main functions of immune checkpoints

Checkpoints	Functions	Reference
PD-1	Binding with its ligand PD-L1/PD-L2 of target cells, counteracting CD80–CD28 signaling transduction of CTLs.	[171]
CTLA-4	Interferes with CD8 T-cell movements and the ability to form stable conjugates with APCs, thus reducing the contact time between cells	[172]
LAG-3	Binding with CD3 in the TCR complex and inhibiting its signal transduction, leading to reduced T cell proliferation and cytokine production	[173]
TIM-3	The switching of the binding TIM-3 and Bat3 or Fyn, further inhibiting upstream TCR signaling	[174]
TIGIT	Inhibiting TCR signaling by binding with CD155 of APCs	[175]
ICOS	Weaking the function of CD28 signaling by binding with CD275 of APCs	[176]

Fig. 1 Schematic representation of the differentiation of T cells from common lymphoid progenitors.

Schematic representation of the differentiation of T cells from common lymphoid progenitors. Common lymphoid progenitor (CLP) cells, which originate in the red bone marrow, give rise to immature precursor T cells. These precursor cells are initially double-negative for both TCR and CD proteins. Thymic chemotactic factors, such as thymotaxin, thymosin, and thymopoietin, guide these double-negative precursor T cells from the bloodstream into the thymus. Within the thymus, thymic cells present MHC I and II molecules to the developing T cells, prompting the expression of TCR and CD proteins. This interaction ensures positive selection, which

leads to the survival of T cells that can bind MHC molecules with at least weak affinity. T cells that recognize MHC I differentiate into $CD8^+$ T cells, while those recognizing MHC II develop into $CD4^+$ T cells. Furthermore, $CD4^+$ T cells may differentiate into specialized subsets such as Th cells or Treg cells, depending on the presence of specific cytokines and stromal signals. **Abbreviations:** CLP, common lymphoid progenitor; TCR, T-cell receptor; MHC, major histocompatibility complex; CD, cluster of differentiation; Th, T-helper; Treg, T-regulatory.

Fig. 2 Schematic representation of T cell activation upon recognition of antigenic peptides.

The variable (V) regions of the α and β chains of the TCR specifically recognize and bind to antigenic peptides presented by MHC I molecules on target cells. This interaction is enhanced by the co-receptor CD8, which binds to both the TCR and MHC I, stabilizing the TCR–CD3 complex at the MHC–peptide interface. This stable interaction leads to the phosphorylation of ITAMs within the CD3 subunit of the TCR complex. The phosphorylation of ITAMs activates downstream signaling cascades that result in the activation of transcription factors such as NF-κB, NFAT, and AP-1, ultimately driving the proliferation and effector function of the $CD8^+$ T cell. These effector functions include cytokine secretion and the generation of cytotoxic molecules such as perforin and Granzyme B. **Abbreviations:** TCR, T-cell receptor; MHCI, major histocompatibility complex class I; CD, cluster of differentiation; ITAM, immunoreceptor tyrosine-based activation motif; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NFAT, nuclear factor of activated T-cells; AP-1, activator protein 1.

Fig. 3 Mechanisms by which CTLs mediate BBB damage.

(A) **Perforin/Granzyme Cytotoxicity**: CTLs release perforin and granzyme B, inducing apoptosis of brain microvascular endothelial cells. (B) **Cytokine Signaling**: IFN-γ and TNF-α from CTLs activate JAK/STAT and NF-κB in endothelial cells, downregulating tight junction proteins. (C) **Chemokine-Mediated Trafficking**: CTL-derived CXCL10 and CCL5 establish chemotactic gradients, recruiting CTLs and bystander leukocytes via CXCR3 and CCR5. **Abbreviations:** CTL, cytotoxic T lymphocyte; BMEC, brain microvascular endothelial cell; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; JAK, Janus kinase; STAT, signal transducer and activator of transcription; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ICAM-1, intercellular adhesion molecule-1;

VCAM-1, vascular cell adhesion molecule-1; MMP, matrix metalloproteinase; CXCL10, C-X-C motif chemokine ligand 10; CCL5, C-C motif chemokine ligand 5; CXCR3, C-X-C motif chemokine receptor 3; CCR5, C-C motif chemokine receptor 5.

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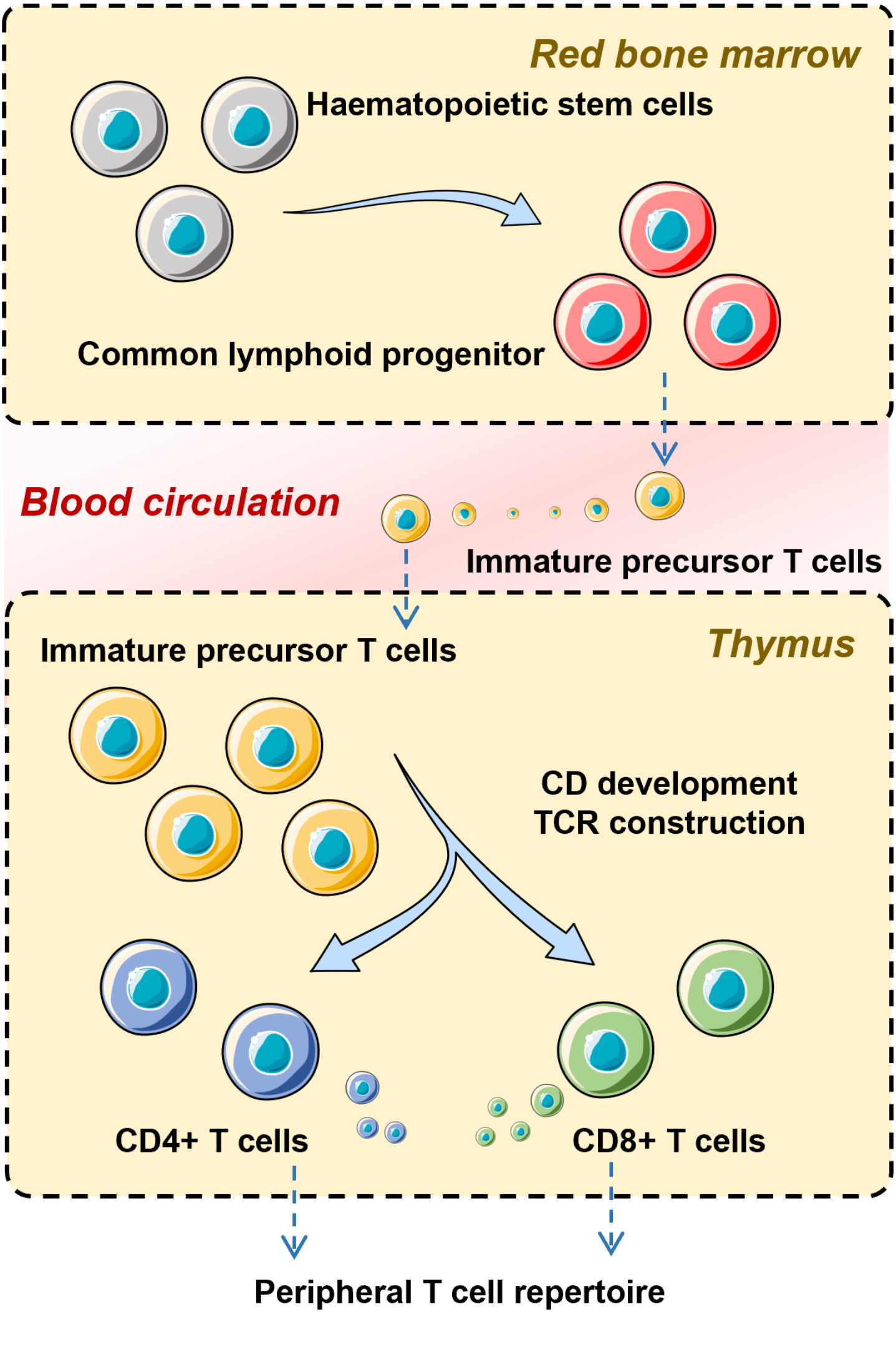
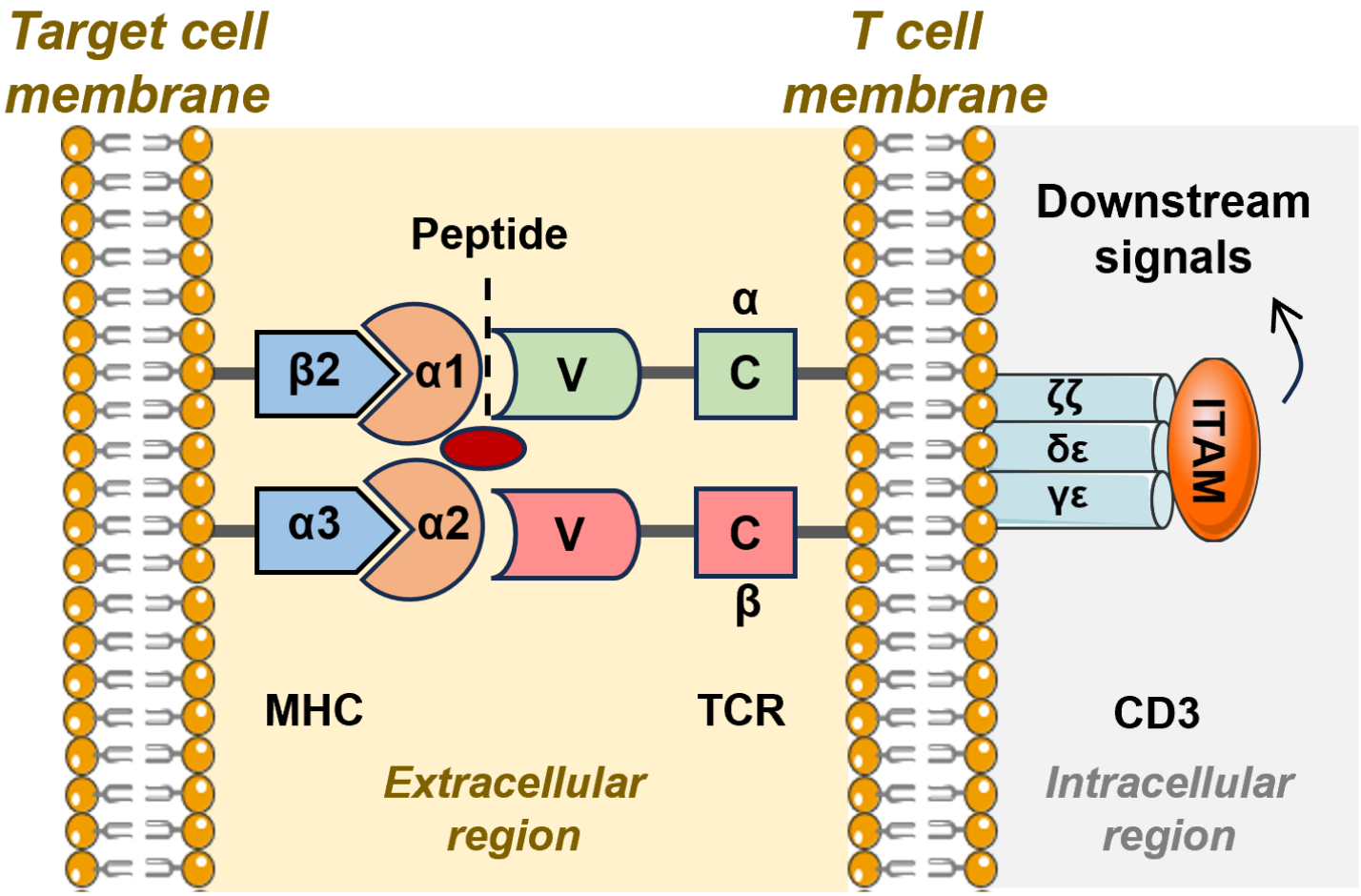
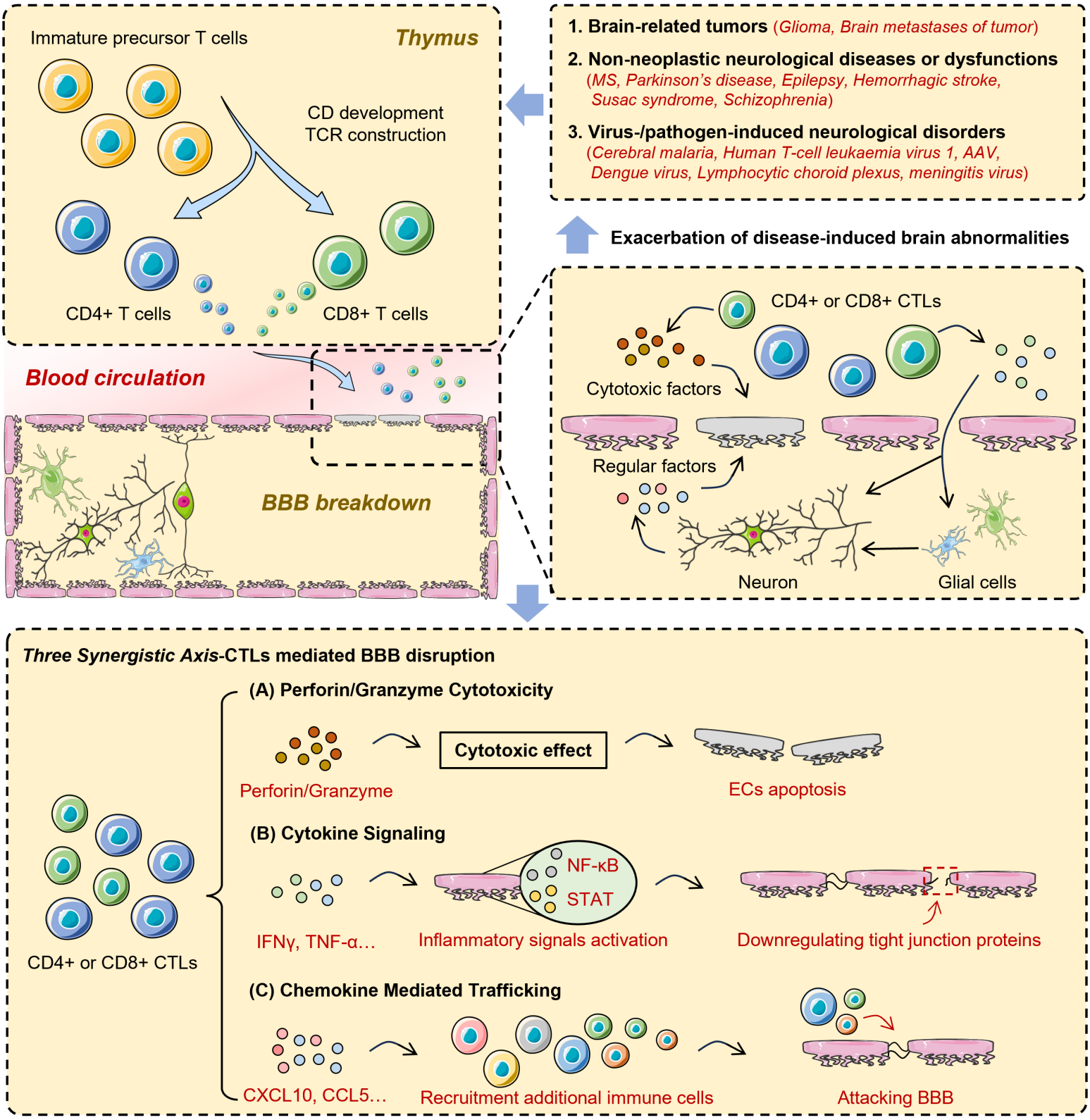

Red bone marrow
Haematopoietic stem cells
Common lymphoid progenitor
Blood circulation
Immature precursor T cells
Immature precursor T cells
Thymus
CD development
TCR construction
CD4+ T cells
CD8+ T cells
Peripheral T cell repertoire


Target cell membrane
T cell membrane
Peptide
β2
α1
V
α
C
α3
α2
V
C
β
Downstream signals
ζζ
δε
γε
ITAM
MHC
TCR
CD3
Extracellular region
Intracellular region


Immature precursor T cells
Thymus
CD development
TCR construction
CD4+ T cells
CD8+ T cells
Blood circulation
BBB breakdown
1. Brain-related tumors (Glioma, Brain metastases of tumor)
2. Non-neoplastic neurological diseases or dysfunctions
(MS, Parkinson's disease, Epilepsy, Hemorrhagic stroke, Susac syndrome, Schizophrenia)
3. Virus-/pathogen-induced neurological disorders
(Cerebral malaria, Human T-cell leukaemia virus 1, AAV, Dengue virus, Lymphocytic choroid plexus, meningitis virus)
Exacerbation of disease-induced brain abnormalities
CD4+ or CD8+ CTLs
Cytotoxic factors
Regular factors
Neuron
Glial cells
Three Synergistic Axis-CTLs mediated BBB disruption
(A) Perforin/Granzyme Cytotoxicity
Perforin/Granzyme
Cytotoxic effect
ECs apoptosis
(B) Cytokine Signaling
IFNγ, TNF-α…
NF-κB
STAT
Inflammatory signals activation
Downregulating tight junction proteins
(C) Chemokine Mediated Trafficking
CXCL10, CCL5…
Recruitment additional immune cells
Attacking BBB
CD4+ or CD8+ CTLs

Supplementary Table 1 The discovery process of the BBB

Name	Year	Contribution	Reference
Ridley Humphrey	1695	The low permeability of small cerebral vessels	[162]
Ehrlich Paul	1885	The isolating between brain and bloodstream	[13]
Lewandowsky Max	1909	Terming this new concept as a German name bluthirnschranke	[163]
Goldmann Edwin Ellen	1909	Only the brain and the spinal cord can be stained by Evans blue injected in ventricles	[14]
Stern Lina & Raymond Gautier.	1921	Naming it as “barrière hémato-encéphalique” in French, and then translated into BBB	[15]
Stern Lina	1929	the BBB was not mature during embryogenesis	[164]

Supplementary Table 2 The main functions of components of BBB

Components	Functions	Reference
Endothelial cells	Endothelial cells are tightly interconnected, forming distinct lumenal and abluminal membrane compartments	[165]
Pericytes	Pericytes are embedded in the basement membrane and lie abluminal to the endothelial cells, and in close communicate with endothelial cells	[166, 167]
Astrocytes	Astrocytes surround blood vessels in the brain, serving as the interface between neurons and endothelial cells	[168]
Tight junctions	Tight junctions reside between endothelial cells, serving as the main functional components in sustaining the permeability barrier and controlling tissue homeostasis	[169]
Adherent junctions	Adherent junctions are fundamental for the integrity of BBB, any change of adherens junctions may disrupt inter-endothelial cell connections	[170]

Supplementary Table 3 The main functions of immune checkpoints

Checkpoints	Functions	Reference
PD-1	Binding with its ligand PD-L1/PD-L2 of target cells, counteracting CD80–CD28 signaling transduction of CTLs.	[171]
CTLA-4	Interferes with CD8 T-cell movements and the ability to form stable conjugates with APCs, thus reducing the contact time between cells	[172]
LAG-3	Binding with CD3 in the TCR complex and inhibiting its signal transduction, leading to reduced T cell proliferation and cytokine production	[173]
TIM-3	The switching of the binding TIM-3 and Bat3 or Fyn, further inhibiting upstream TCR signaling	[174]
TIGIT	Inhibiting TCR signaling by binding with CD155 of APCs	[175]
ICOS	Weaking the function of CD28 signaling by binding with CD275 of APCs	[176]