Keystone Epitope Theory: Implications for the Investigation of Multiple Sclerosis

Simon Mallal[1,2], Amir Asiaee[3], Natalie Mallal[4] and Elizabeth Phillips[1,2]

[1] Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA

[2] Institute for Immunology and Infectious Diseases, Murdoch University, Perth, Western Australia

[3] Department of Biostatistics, Vanderbilt University Medical Center, Nashville, Tennessee, USA

[4] School of Arts and Sciences, Vanderbilt University, Nashville, Tennessee, USA

Abstract

Multiple sclerosis (MS) has long presented a paradox: a strong and reproducible association with Epstein–Barr virus (EBV) exposure despite EBV's near-universal prevalence. Large prospective cohorts show that EBV seroconversion precedes MS onset and is associated with a marked increase in risk [1], while earlier work links risk to prior infectious mononucleosis and to elevated anti–EBV nuclear antigen 1 (EBNA1) antibody responses [2] [3]. Yet EBV is clearly not sufficient, implying additional "gates" governed by host genetics, developmental timing, and tissue context.

Keystone Epitope Theory (KET) proposes that persistent, human-adapted pathogens such as EBV imprint high-gain immune priorities toward constrained peptide–HLA geometries ("keystone epitopes"), creating plausible vulnerabilities when modified self or foreign antigens accidentally mimic those geometries in the right HLA and niche [4] [5] [6] [7] [8] [9] [10]. Applied to MS, KET reframes EBV association as a conditional recall problem: an EBV-trained effector program, often focused on latent antigens such as EBNA1 and deployed in B-cell follicles by cytotoxic T cells (including class II–restricted cytotoxic CD4 T cells), may be redeployed against CNS targets when mimic density and inflammatory context overcome local inhibitory set points. This model can accommodate partial penetrance, epitope spreading, and the strong effects of HLA-DRB1*15:01.

The review summarizes key evidence for EBV-focused immunity in MS, highlights newer findings on EBNA1-to-CNS molecular mimicry [11] [12] and host–virus genetic convergence [13], and then proposes a practical investigational workflow: lesion-anchored, TCR-forward mapping to identify the keystone epitope and restricting HLA first, followed by a targeted search for homologous CNS mimics, including post-translationally modified (PTM) peptides.

Key messages

- The epidemiological evidence strongly supports EBV exposure as a near-necessary antecedent in most MS cases studied to date, but the mechanism remains the central gap [1] [14].
- EBNA1-focused immunity is a recurring signal across serology, HLA interactions, and molecular mimicry datasets [2] [15] [11].
- KET compresses the mechanistic search space by prioritizing the "time and site of the crime": expanded lesion/CSF TCR clonotypes and their restricting peptide–HLA surfaces.
- A practical KET workflow is TCR-forward: identify restricting HLA and keystone epitope first, then search for CNS mimics and relevant PTMs. This avoids the otherwise unbounded PTM search space.

1. Problem statement: the unmet mechanistic need

MS risk is shaped by a familiar triad: genetics (particularly HLA class II), environment (vitamin D and UV exposure, smoking), and infection history. EBV sits at the intersection. However, the field still lacks a tractable, testable map that links EBV immunity to the defining pathology of MS: compartmentalized CNS inflammation, blood–brain barrier perturbation, demyelination, and progressive neurodegeneration.

KET's claim is not that EBV is simply a trigger, but that EBV acts as a calibrator of immunodominance hierarchies. In this view, autoimmunity can reflect a failure of prioritization under constraints rather than an indiscriminate collapse of tolerance [5] [6] [10]. The practical question becomes: which EBV-trained clonotypes, recognizing which peptide–HLA geometries, are executing injury in the CNS niche? The overall KET-MS logic, including the four-condition gate and the post-initiation consequences, is summarized in Figure 1.

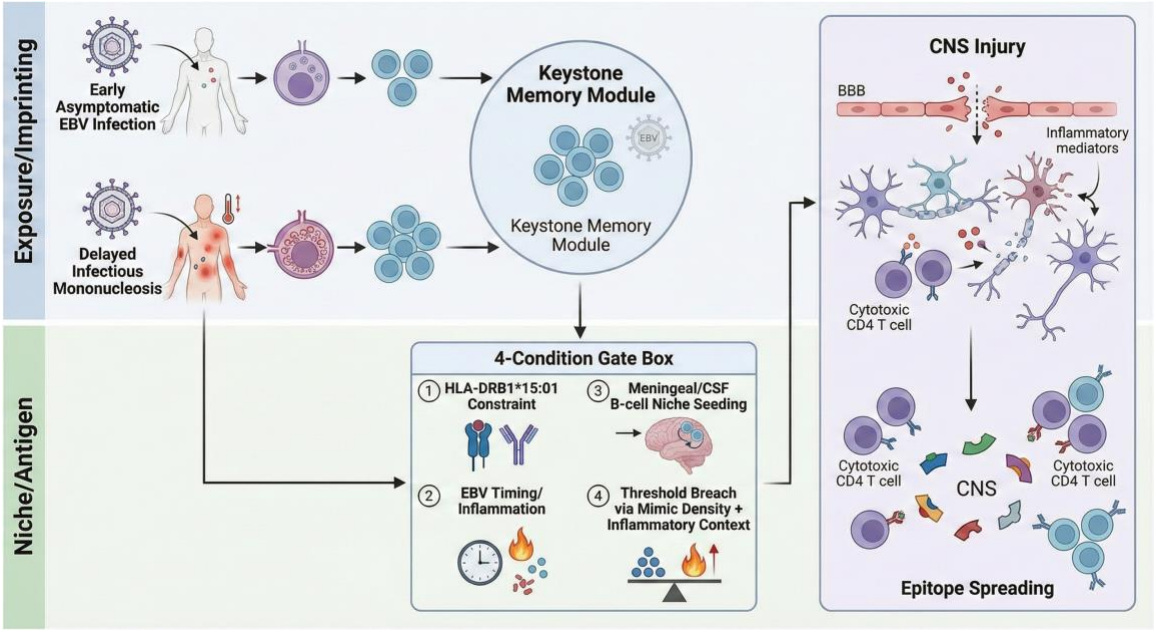

Figure 1. Keystone Epitope Theory framework for EBV-associated multiple sclerosis. Conceptual overview illustrating how Epstein–Barr virus (EBV), as a human-adapted keystone organism, imprints high-gain immune memory toward constrained peptide–HLA geometries. Early, tolerogenic EBV acquisition calibrates durable equilibrium, whereas delayed or inflammatory primary infection biases memory allocation and regulatory set points. In genetically permissive hosts, subsequent presentation of a keystone-mimetic peptide in a CNS-relevant niche can breach local inhibitory thresholds, initiating tissue injury and downstream epitope spreading.

With that map in place, the next sections lay out the empirical constraints and suggest how they can be translated into a testable discovery workflow.

What / Why / How (KET lens)

Table 1. What / Why / How framing of Keystone Epitope Theory applied to multiple sclerosis.

Question	KET interpretation	Implication for MS investigation
What is the problem?	We can describe EBV association and HLA risk but cannot yet specify the initiating epitope geometry that converts EBV immunity into CNS injury.	Rather than searching broadly for 'autoantigens'; we could start by mapping lesion/CSF effector receptors to their cognate pMHC.
Why does it arise (development + evolution)?	EBV is a long co-adapted persistent virus. Early-life imprinting tends to occur in a tolerizing milieu; delayed, inflammatory primary infection may distort clonotype selection and regulatory set points.	Treat timing of EBV acquisition (IM vs early asymptomatic infection) as a modifier of the "activation threshold" gate.
How do we fix the knowledge gap?	Identify the keystone epitope first using TCR-forward mapping and restricting HLA, then search for CNS mimics and PTMs that recreate that surface at sufficient density.	A bounded, experimentally falsifiable workflow that can converge on a seed epitope before epitope spreading obscures the trail.

2. Empirical constraints: what the data force us to explain

2.1 EBV exposure and MS risk precede disease onset

Prospective cohorts demonstrate that EBV seroconversion precedes MS onset and is associated with a large increase in MS risk, supporting temporality and specificity [1]. Earlier prospective studies have shown that elevated anti-EBV antibody titers (including EBNA responses) can precede MS diagnosis[2], and analyses of US military serum repositories support anti-EBV antibodies as informative serologic markers.[16]

Infectious mononucleosis (symptomatic delayed primary EBV infection) is associated with a consistent approximate two-fold increase in MS risk in meta-analyses [3] [17] and persists over decades in large population datasets [18].

Several lines of recent work provide a plausible mechanistic link between delayed primary EBV infection and subsequent establishment of intrathecal immune niches relevant to MS pathogenesis. Symptomatic primary EBV infection has been reported to generate B-cell subsets that acquire CNS-homing behavior and recruit inflammatory T cells, offering a potential route from delayed primary infection to formation of an intrathecal antigen-amplifier compartment [19]. Consistent with this directionality, EBV persistence in B cells has been associated with expansion and functional bias of $CXCR3^{+}$ memory B-cell subsets with brain-homing features and enhanced anti-EBNA1 plasma-cell differentiation in MS contexts [20].

2.2 EBNA1 immunodominance and risk stratification with HLA

Multiple datasets implicate EBNA1 as a central antigen in the EBV–MS relationship. Elevated EBNA1 IgG titers are associated with increased MS risk, and antibody reactivity to specific EBNA1 regions has shown particularly strong associations [15]. In a population-based case-control cohort, combining HLA-DR risk profiles with EBV serology substantially improved discrimination between MS and controls [21].

2.3 Environment and other modifiers act like 'threshold knobs'

Vitamin D, UV exposure and smoking are reproducible MS modifiers, and plausibly intersect with EBV immune control. A vitamin D response element in the HLA-DRB1*15:01 promoter suggests a direct mechanistic link between vitamin D biology and the major genetic risk locus [22]. Smoking is a clean example of a context-dependent 'threshold knob' (Figure 6): it interacts with MS HLA risk architecture to amplify risk in a manner that fits a gated penetrance model [23]. Smoking has also been associated with higher anti-EBNA1 titers in MS-relevant epidemiologic analyses, aligning with the idea that peripheral inflammatory priming can intensify EBV-linked immune programs [24]. Genomic studies indicate that EBV transcriptional regulators, particularly EBNA2, occupy autoimmune susceptibility intervals including those associated with MS and intersect with host regulatory architecture such as vitamin D receptor occupancy, creating a plausible surface for gene-environment-virus interactions that modulate threshold control rather than acting as single-cause triggers [25] [26]. Beyond HLA, large-scale MS genetic mapping implicates regulatory programs across peripheral immune cells and CNS-resident microglia, consistent with a model in which breach amplification and downstream injury involve both infiltrating lymphocytes and CNS-resident antigen-presenting circuits once the gate is crossed [27].

A further, still under-tested axis is germline diversity in antigen receptors themselves, particularly TCR loci, and how this diversity may interact with EBV strain variation across human populations.

2.4 Germline TCR variation, EBV strain diversity, and discordant coevolution: an underused axis for gating

KET treats HLA as a principal “hardware” constraint, but peptide-HLA recognition is a two-body problem: the other half is the receptor. The germline TCR loci (TRA/TRB/TRG/TRD) contain coding polymorphisms and haplotypic structure that can alter V-gene sequence features (including CDR1/CDR2 chemistry) and bias which peptide–HLA geometries are most easily generated, selected, and expanded [28,29]. This matters for a TCR-forward strategy in MS because “public” or stereotyped TCRs can be partly germline-enabled, not purely antigen-selected, and failure to account for germline TR alleles can distort V-gene assignment and cross-cohort comparisons.

Historically, several candidate-gene and linkage studies have suggested that polymorphisms near TCR loci may contribute to MS susceptibility. These included reported associations or linkage signals involving TCRβ or TCRα/δ markers [30] [31] [32] [33]. However, replication was inconsistent, and multiple cohorts reported no convincing association between MS and TCR gene polymorphisms across α, β, γ, and δ chains [34] [35] [36]. A later synthesis emphasized that TR loci are structurally complex and historically poorly captured by standard marker sets, so the net message is best framed as “not established” rather than “excluded,” with the additional warning that population structure and technical ascertainment may have contributed to both positive and negative findings [37].

However, the feasibility of such studies is now improved. High-throughput repertoire sequencing can infer germline TR alleles directly from AIRR-seq data and has revealed extensive TRB germline variability, including alleles absent from standard references and showing ancestry stratification [38] [39]. Complementary approaches using improved genomic resources and targeted TR locus sequencing continue to expand the known catalog of germline TR alleles, revealing substantial previously unrecognized diversity that was poorly captured by earlier reference sets [40]. More broadly, given the well-established out-of-Africa gradient in human genetic diversity, it remains plausible that germline TR diversity is similarly structured across populations, although current TR-focused datasets do not yet distinguish between founder effects, selection, or coevolution. This broader framing is supported by population-genetic analyses showing a serial founder effect and monotonic loss of genetic diversity with distance from Africa across many human loci [41] [42].

Earlier population surveys also documented dense SNP variation across TCR loci and substantial allele frequency differences among African American, Chinese, Mexican and Northern European samples, with patterns broadly compatible with demography while not excluding selection or co-evolution [43,44].

In parallel, EBV shows pronounced phylogeographic structure and deep type 1/type 2 divergence (largely defined by EBNA2/EBNA3) with linked variation extending into additional loci, including genes involved in B-cell tropism and immunogenic targets [45] [46] [47]. Genome-wide analyses recover geographically stratified EBV subpopulations and enrichment of linked variation in immunogenic genes, consistent with a role for host immune pressure and local adaptation, while also making a key methodological point: it is difficult to disentangle host

demography and sampling structure from true selection in a polymorphic virus [48]. Moreover, genome-to-genome analyses show that human genetic variation can correlate with EBV sequence variation, suggesting that host factors can shape EBV diversity at measurable scales [49]. A related “discordant coevolution” concept has been explicitly proposed in other EBV-associated contexts in admixed populations [50].

Taken together, there are at least three plausible explanations for correlated geography in HLA/KIR, germline TR alleles, and EBV strain variation: founder effects during migrations, simple selection operating on each component without coadaptation, or genuine coevolution and “matched” equilibrium states between host receptors and locally circulating EBV lineages. At present, these explanations cannot be cleanly distinguished, so strong claims should be avoided. However, the general principle has precedent. In Helicobacter pylori, discordant pairing of host ancestry and bacterial ancestry associates with increased gastric inflammation and pre-cancerous lesions, whereas matched host–pathogen ancestry is comparatively benign, implying that disruption of coevolved relationships can modulate disease risk [51].

For MS, KET turns this into a testable modifier of the four-condition gate: not only whether EBV was acquired, but whether the infecting EBV strain’s keystone-relevant epitope geometries align with the host’s HLA and germline TCR landscape in a way that preserves regulated equilibrium. Practically, this argues that TCR-forward mapping of expanded lesion/CSF clonotypes should be paired with germline TR allele calling (to ensure accurate V-gene assignment and enable cross-population analysis) and, where feasible, EBV strain sequencing. A null result would still be informative; a positive result would be mechanistically transformative, because it would define a concrete axis of “discordant coevolution” that can be integrated into the gated model.

3. EBV biology relevant to a keystone model

EBV persists for life in the B-cell compartment with tightly regulated latency programs. The infected memory B-cell pool provides long-term persistence, with periodic transitions through proliferative programs and reactivation. EBNA1 is required for maintenance and segregation of the EBV episome in dividing cells, and its immune visibility is shaped by immune evasion mechanisms that can bias presentation toward class II pathways [14]. The relevant latency programs and where EBNA1 persists across B-cell differentiation are summarized in Figure 2.

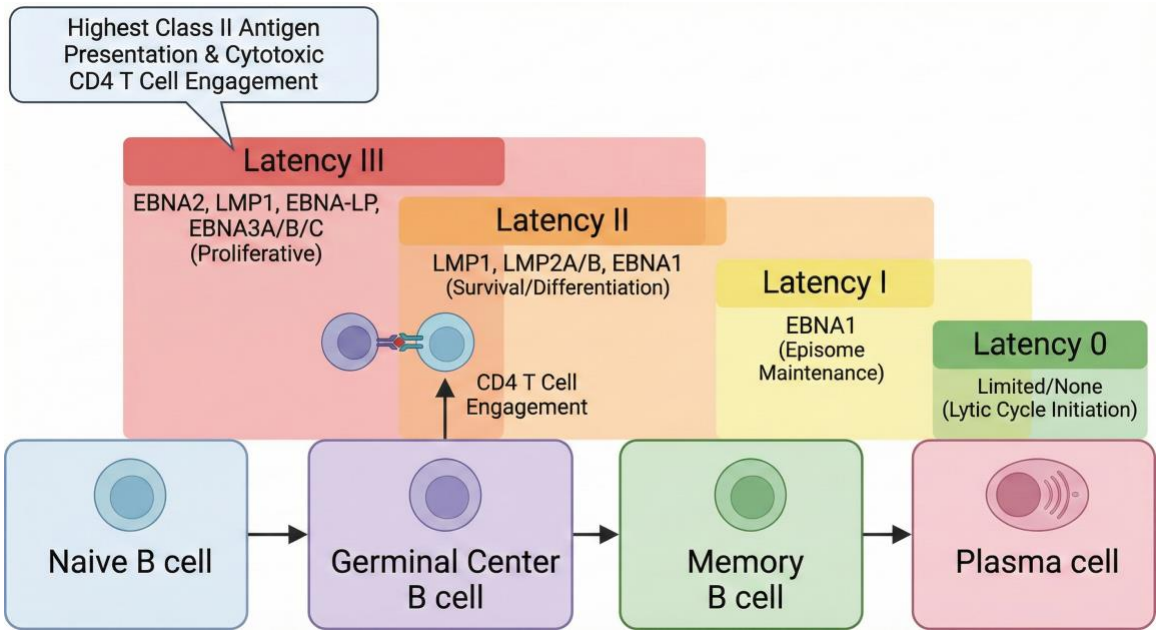

Figure 2. EBV latency programs and immune surveillance across the B-cell lifecycle. Schematic of EBV infection from naïve B cells through germinal center transit to long-lived memory B cells, highlighting restricted latency programs and constitutive EBNA1 expression required for episome maintenance. The figure emphasizes class II antigen presentation within follicular niches and the roles of cytotoxic $CD4^+$ T cells, $CD8^+$ T cells, and NK-cell interoperability in controlling transformed or proliferating EBV-infected B cells.

KET's practical takeaway is that EBV supplies a chronic, anatomically organized antigen source in follicular niches. This is where cytotoxic control is biologically meaningful. For MS, a central unresolved question is whether follicular EBV control is mediated predominantly by EBV-specific cytotoxic CD4 T cells (class II–restricted), by CD8 T cells, or by a coordinated program involving both, and whether these effector pathways can be misdirected by peptide mimicry. This sets up the next question: where does this B-cell–centered persistence intersect with the CNS compartment?

4. The CNS compartment: B cells, follicles, and oligoclonal IgG

CSF oligoclonal bands (OCBs) are present in the majority of MS cases and reflect clonal expansion of intrathecal B cells and plasmablasts [52] [53]. Meningeal lymphoid aggregates and follicle-like structures have been described in a subset of MS cases and provide a plausible intrathecal 'antigen-amplifier' niche. In secondary progressive MS, their presence has been linked to more severe cortical pathology and earlier disease onset, supporting the niche-seeding logic that the gated KET model later operationalizes [54]. Two issues are often conflated: (i) whether meningeal follicle-like structures occur and associate with cortical injury, and (ii) whether EBV is consistently detectable within those structures and adjacent CNS tissue. A widely cited positive neuropathology study reported EBV-infected B cells and plasma cells in MS

brains and identified meningeal follicles as major sites of EBV persistence [55]. However, the extent to which EBV-infected B cells are consistently present in CNS tissue remains contested across laboratories [56,57].

Claims that OCBs are predominantly EBV-specific have been reported [58] [59], but other studies support a polyspecific intrathecal antibody response and caution against assuming EBV specificity of OCBs as a general rule [60]. The KET implication is straightforward: antibody specificity is informative, but the decisive step for tissue injury is more likely the effector TCR recognition event at the CNS site.

5. The effector arm: EBV-specific T cells and the special case for cytotoxic CD4

Multiple studies show that MS CSF contains expanded, patient-private T-cell clones, and that T cells in CSF can recognize autologous EBV-transformed B cells [61]. Primary EBV infection can elicit oligoclonal expansions of EBV-specific CD4 T cells with cytotoxic effector programs detectable at the single-cell level, consistent with a genuine class II–restricted CD4 cytotoxic arm rather than CD4 'help' alone [62]. EBNA1-reactive cytolytic CD4 clones have also been shown to inhibit proliferation of newly EBV-infected B cells via HLA-DR–restricted recognition early after infection [63]. These observations provide direct biological support for the KET premise that a cytotoxic, EBV-trained class II–restricted program exists and could become pathogenic if a CNS peptide-HLA surface functionally mimics a keystone EBV geometry in an inflammatory context. Recent work in paired blood/CSF analyses has reported that some expanded CSF T-cell clones are specific for autologous EBV-infected B cells [64].

The fact that class II–restricted CD4 T cell cytotoxicity to EBV can target infected B cells [63] and shape germinal-center control is relevant to MS because the follicular niche is a plausible 'keystone niche' that is relatively enriched for class II antigen presentation and may be less accessible to CD8 surveillance.

A well-established parallel literature shows that active MS lesions often contain dominant clonal expansions of CD8 T cells [65], and intrathecal repertoire studies have reported enrichment of EBV-reactive CD8 TCR sequences in MS CSF [66]. This does not weaken the KET workflow; it strengthens the operational point. The 'T cells at the time and site of the crime' may be CD8 and/or cytotoxic CD4 T cells, so receptor-first mapping should start agnostically from expanded lesion/CSF clonotypes and only then move upstream to keystone-epitope identification and CNS mimic discovery.

KET emphasizes that these cytotoxic CD4 programs are not inherently autoreactive. The risk emerges when the same TCR geometries are engaged by CNS-presented mimics in the correct HLA context, and when local inhibitory set points (PD-1/PD-L1, CTLA-4, regulatory networks) are exceeded [5,10]. Notably, EBV-infected B cells in MS brain samples have been reported to express PD-L1, suggesting a local immune-evasion microenvironment that could distort control and

increase antigen burden [67]. A follicle-centric model for cytotoxic CD4⁺ control of EBV-infected B cells, and how the same program could be misdirected to CNS targets when the threshold is breached, is illustrated in Figure 3.

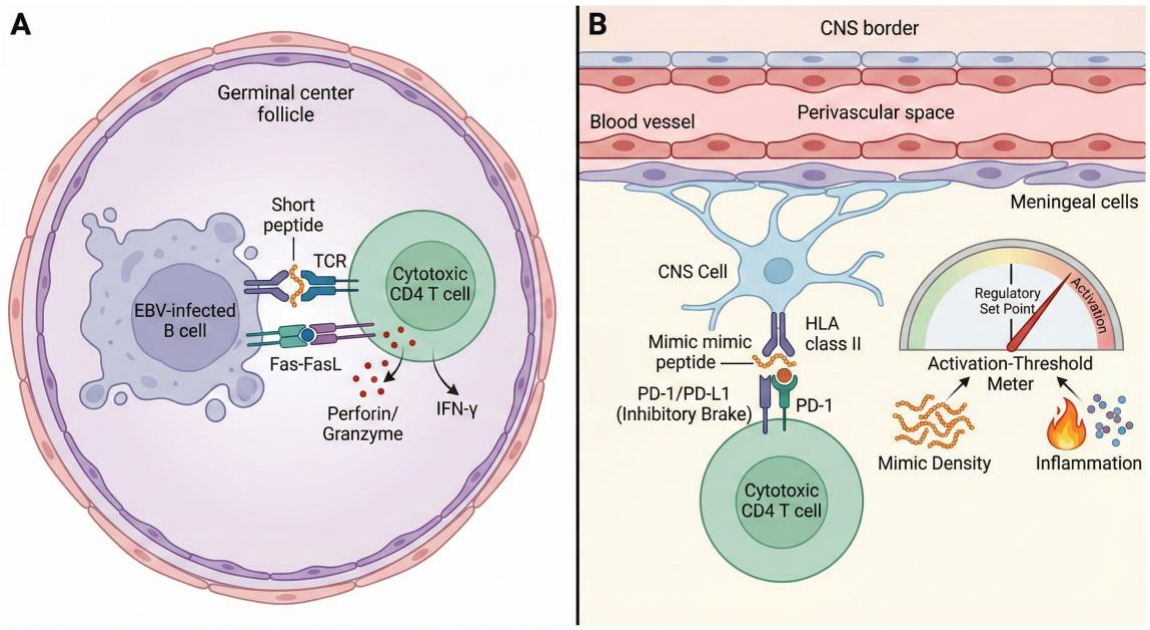

Figure 3. Cytotoxic CD4⁺ T-cell control of EBV-infected B cells and potential misdirection to the CNS. Model depicting class II–restricted cytotoxic CD4⁺ T cells as key effectors controlling EBV-infected B cells within germinal centers and ectopic follicles. Under permissive conditions, the same effector program could be redeployed against CNS targets that present a keystone-mimetic peptide–HLA complex, thereby contributing to blood–brain barrier disruption and focal tissue injury.

As shown in Figure 3, the key open mechanistic question is what CNS-presented peptide-HLA surfaces could plausibly engage these EBV-trained receptors. In KET terms, molecular mimicry is the experimentally tractable bridge once the relevant TCR and restricting HLA are known.

6. Molecular mimicry: moving from EBV immunity to CNS targets

Among proposed mechanisms linking EBV immunity to CNS injury, receptor-anchored molecular mimicry currently has some of the strongest direct mechanistic support. A landmark study isolated CSF-derived monoclonal antibodies from MS and demonstrated molecular mimicry between EBNA1 and the CNS protein GlialCAM, supported by structural data and functional readouts [11]. Follow-on work indicates that combined antibody reactivity to EBNA1 and GlialCAM-related mimics can differentiate MS patients from controls and may help define an EBNA1-mimicry–linked serologic endotype [12].

Independent lines of evidence propose EBNA1 mimicry with additional CNS antigens, including anoctamin-2 (ANO2), where immune reactivity to ANO2 associates with MS risk and interacts with HLA and EBNA1 antibody levels [68]. At minimum, these findings make EBNA1 a rational starting antigen for KET-guided epitope mapping in MS. Representative EBNA1-to-CNS mimicry candidates motivating this search strategy are summarized in Figure 4.

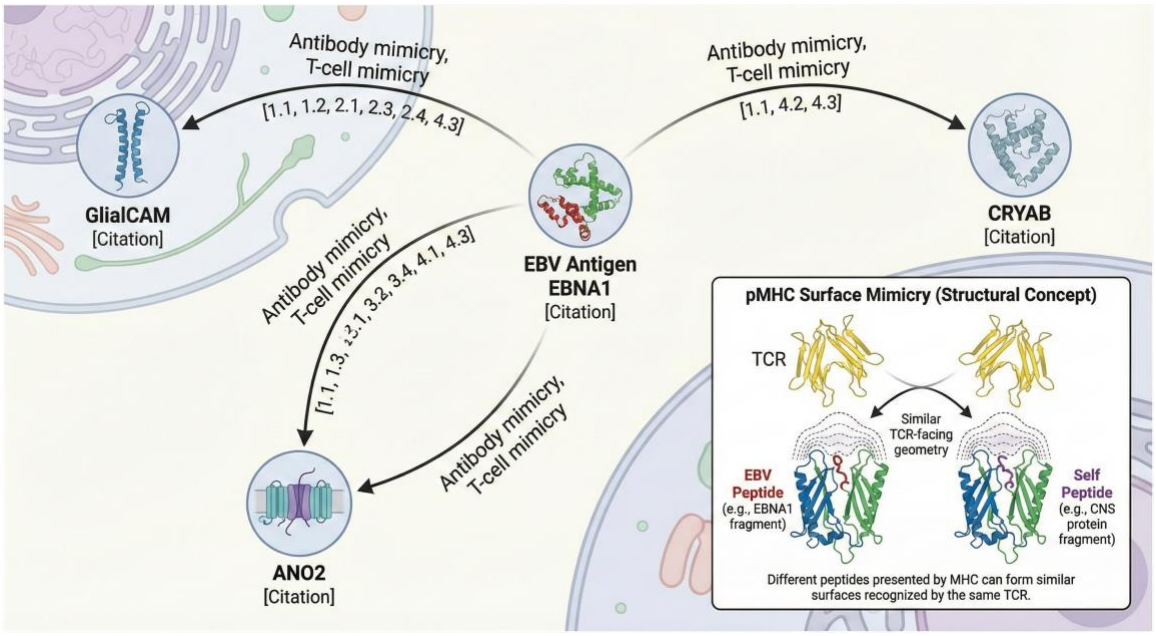

Figure 4. Integration of EBV-linked epidemiologic signals, EBNA1-focused serology, and intrathecal oligoclonal IgG in MS.
Schematic linking EBV exposure and EBNA1-directed humoral immunity to MS risk, alongside intrathecal oligoclonal IgG as a marker of compartmentalized CNS humoral responses. Candidate examples of EBNA1-to-CNS molecular mimicry are shown to illustrate how keystone-trained responses might cross-recognize CNS targets when local inhibitory thresholds are exceeded, while remaining insufficient for disease in most hosts.

These receptor-anchored mimicry datasets sharpen what may be necessary, but they still do not explain why most EBV-seropositive carriers of MS-associated HLA haplotypes never develop MS. KET addresses this gap by treating MS as a gated recall event that depends on niche seeding and a breach of local inhibitory thresholds, rather than on exposure alone.

7. KET applied to MS: a four-condition gate

KET is most useful when operationalized as a gated model. A workable MS version is a four-condition gate, analogous to conditional recall models in HLA-associated drug hypersensitivity:

1. HLA constraint (the ‘hardware’): MS risk haplotypes (especially HLA-DRB1*15:01) constrain the peptide–HLA surfaces that can be stably presented and recognized.

2. Keystone imprinting (the ‘training’): timing and inflammatory context of primary EBV infection shape clonotype selection, regulatory calibration, and tissue homing [3].
3. Niche seeding (the ‘where’): EBV persistence in B-cell niches, including intrathecal B-cell compartments and follicle-like structures, provides chronic antigen presentation opportunities.
4. Threshold breach (the ‘why now’): sufficient mimic density, together with inflammatory context, overrides niche-specific inhibitory set points, enabling EBV-trained effector programs to attack CNS targets; once initiated, epitope spreading obscures the initiating seed.

Operationally, the gate must be realized in an anatomical niche. In MS, the blood-brain barrier (BBB) and meningeal interface provide a plausible “antigen amplifier,” where EBV-persisting B-cell lineages, follicle-like aggregates, and local antigen presentation can create the density and context required to exceed a niche-specific inhibitory set point (Figure 5B).

Together, the four conditions provide a plausible explanation for partial penetrance: EBV exposure and HLA risk are common, but MS may require a permissive imprinting history, a CNS-relevant niche, and a threshold-breach event (Figure 5A–B). Once injury begins, compartmentalization and epitope spreading broaden the apparent antigenic repertoire and obscure the initiating seed (Figure 8A).

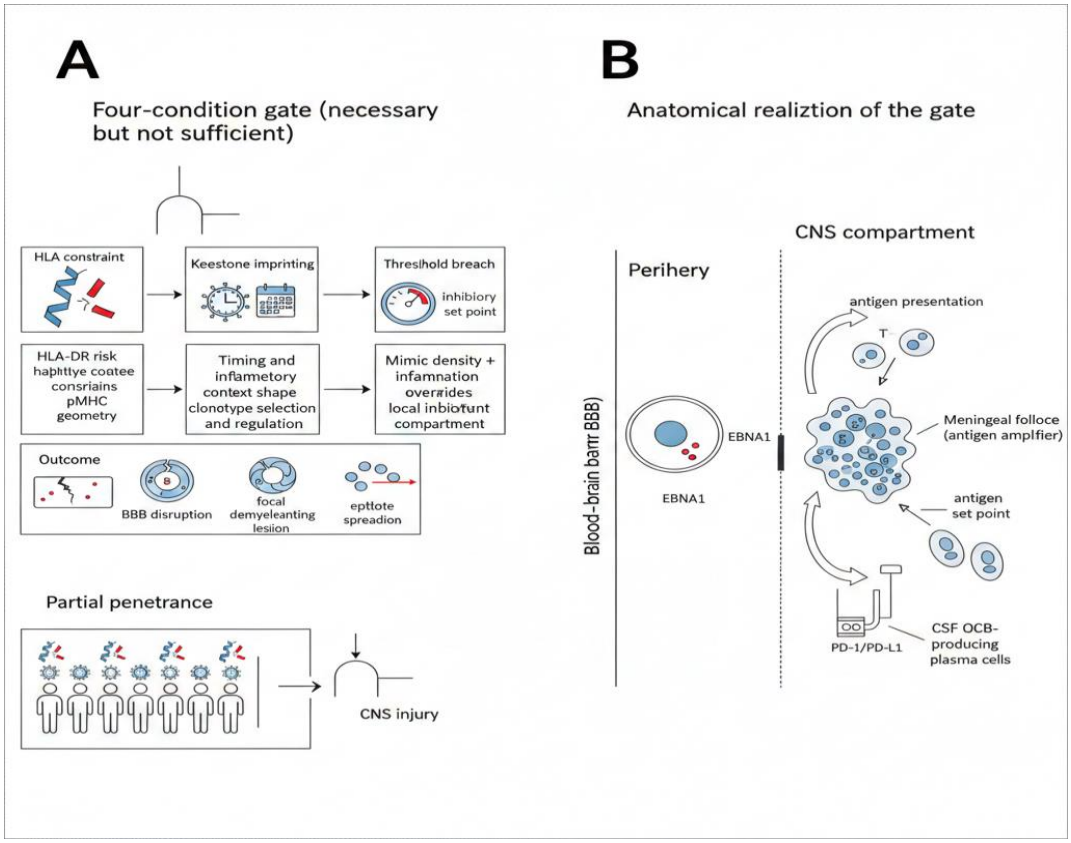

Figure 5. KET applied to MS as a four-condition gate at the CNS border.
(A) Keystone Epitope Theory operationalized as a four-condition gate: (1) HLA constraint defines permissible peptide-HLA geometries; (2) keystone imprinting during primary EBV infection calibrates clonotype selection, regulation, and homing; (3) niche seeding establishes persistent antigen access within B-cell compartments; and (4) threshold breach occurs when keystone-mimetic targets and inflammatory context exceed local inhibitory set points, triggering blood-brain barrier disruption and focal CNS injury. The model accounts for partial penetrance by requiring all four conditions, rather than EBV exposure and HLA risk alone. **(B)** Anatomical schematic illustrating how EBV persistence in memory B cells can become "actionable" at the blood-brain barrier and meningeal interface via follicle-like antigen amplification, antigen presentation to T cells, and intrathecal plasma-cell responses that yield CSF oligoclonal IgG.

7.1 Candidate 'threshold knobs' and testable predictions

In KET terms, these factors function less like independent causes and more like tunable parameters that shift the probability of crossing a niche-specific inhibitory set point. Table 2 lists concrete, testable 'threshold knob' predictions; a simplified schematic is shown in Figure 6.

Table 2. Candidate 'threshold knobs' that modulate the probability of gate breach in KET-MS.

Modifier	KET interpretation	Testable prediction (examples)
Vitamin D/UV	Raises inhibitory and/or central tolerance calibration; interacts with HLA-DRB1*15:01 promoter regulation.	Low vitamin D states have been associated with lower activation thresholds for keystone-mimetic responses and altered Treg calibration [22].
Smoking	Shifts inflammatory tone; may increase EBV antigen load or broaden responses. Gene–environment interaction data show particularly strong risk amplification in smokers carrying HLA-DRB1*15:01 and lacking HLA-A*02, reinforcing the premise that HLA is necessary but context modulates penetrance [23]. Smoking has also been reported to interact with EBV infection markers in MS development [69].	Smoking associates with higher anti-EBNA1 titers and higher probability of threshold breach under the same HLA background [24].
EBV strain / EBNA1 polymorphisms	Alters the keystone epitope geometry and binding affinity landscape.	Specific EBNA1 variants may preferentially generate keystone-mimetic surfaces or higher antigen loads in DR15 backgrounds [70] [71].
Checkpoint tone (PD-1/PD-L1)	Controls local inhibitory set point within follicles and CNS borders.	High PD-L1 expression on EBV-infected B cells has been reported and may track with higher antigen

		burden and altered T-cell phenotypes in lesions [67].
Age / delayed primary infection	First imprint occurs in a more inflammatory milieu with different clonotype selection. Recent mechanistic work suggests that symptomatic primary EBV infection can generate CNS-homing B-cell subsets that recruit inflammatory T cells, providing a plausible 'Condition 3' bridge from infectious mononucleosis timing to intrathecal niche formation.	History of IM associates with broader EBNA1 epitope recognition and higher risk in specific HLA strata [3 21].

A further, currently under-tested modifier is host-virus phylogeographic discordance combined with germline TCR variation, which could shift keystone epitope recognition and inhibitory set points without changing EBV serostatus (Section 2.4).

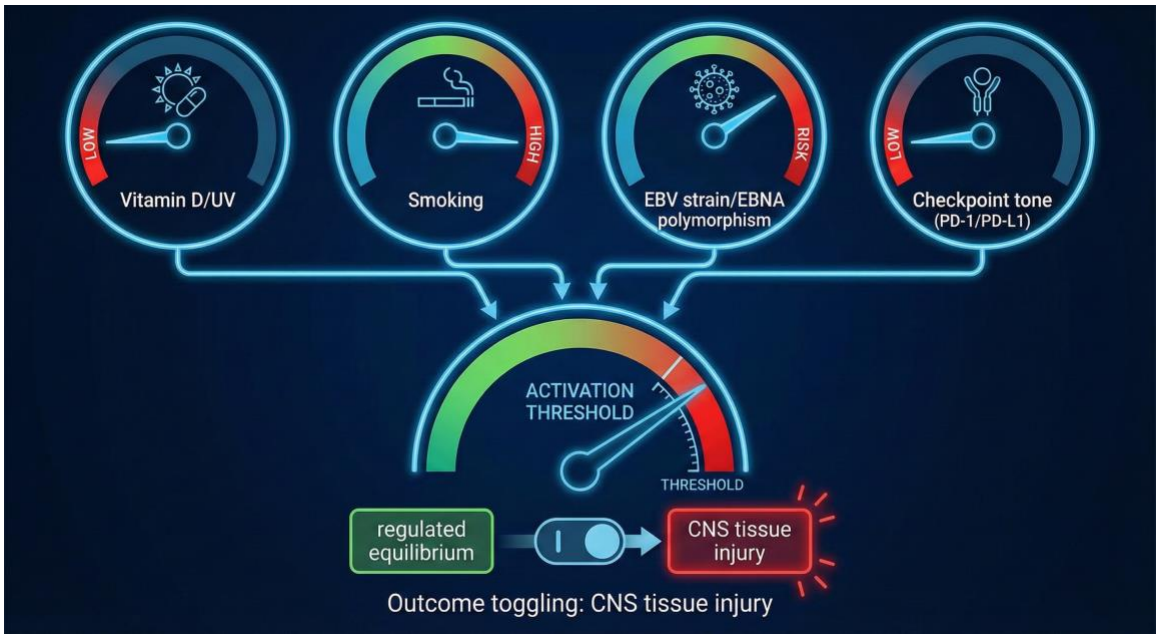

Figure 6. Threshold logic and modifiers of keystone-driven CNS injury. Illustration of how "threshold knobs" (vitamin D/UV, smoking, EBV strain/EBNA1 polymorphisms, checkpoint tone, and age at primary infection) modulate the probability that the four-condition gate is crossed, shifting a regulated EBV equilibrium state toward tissue-destructive recall in a CNS-relevant niche.

8. Practical investigational workflow: lesion-anchored, TCR-forward mapping

The central methodological proposal is to invert the usual approach to autoantigen discovery. Rather than starting with a catalog of plausible CNS proteins and then hunting for responses, KET starts with the effector receptors at the site of injury and asks what they see. The resulting lesion-anchored, TCR-forward workflow is outlined in Figure 7. Because post-initiation epitope spreading rapidly reduces identifiability of the initiating seed, the approach prioritizes early sampling and receptor-first mapping (Figure 8A).

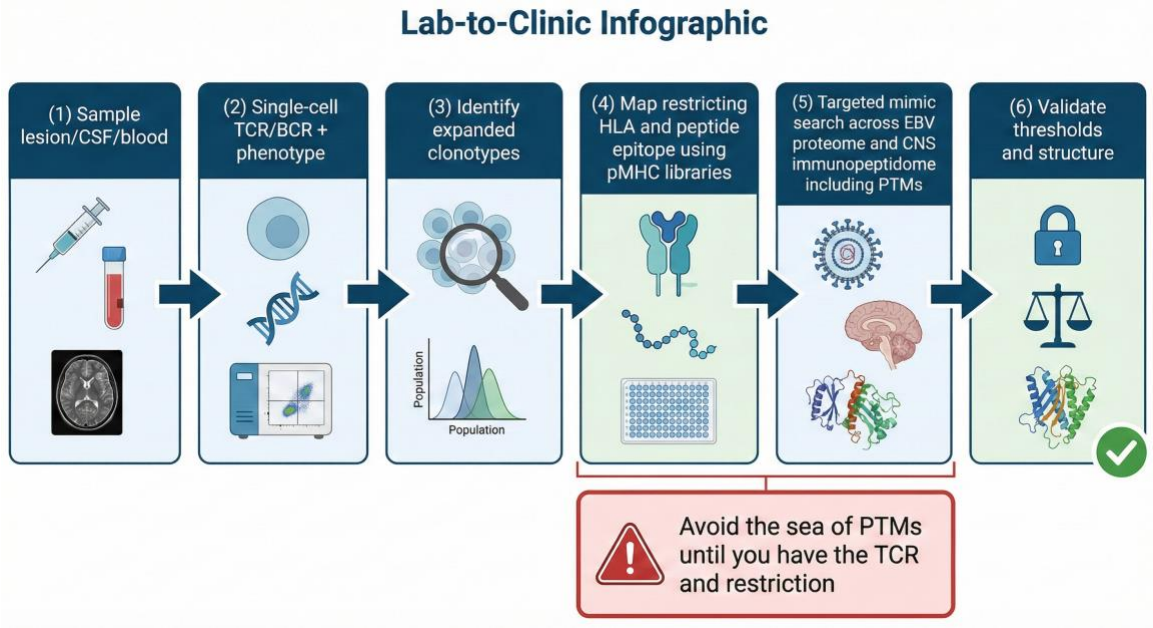

Figure 7. Lesion-anchored, TCR-forward workflow for identifying initiating autoantigens. Experimental roadmap for keystone epitope discovery in MS. Expanded T-cell clonotypes are isolated from lesions or CSF, their HLA restriction and cognate peptides are defined using TCR-forward mapping, and only then are viral and self-immunopeptidomes searched for structural mimics. This ordering is designed to avoid false attribution from the large background of post-translational modification and secondary epitope spreading.

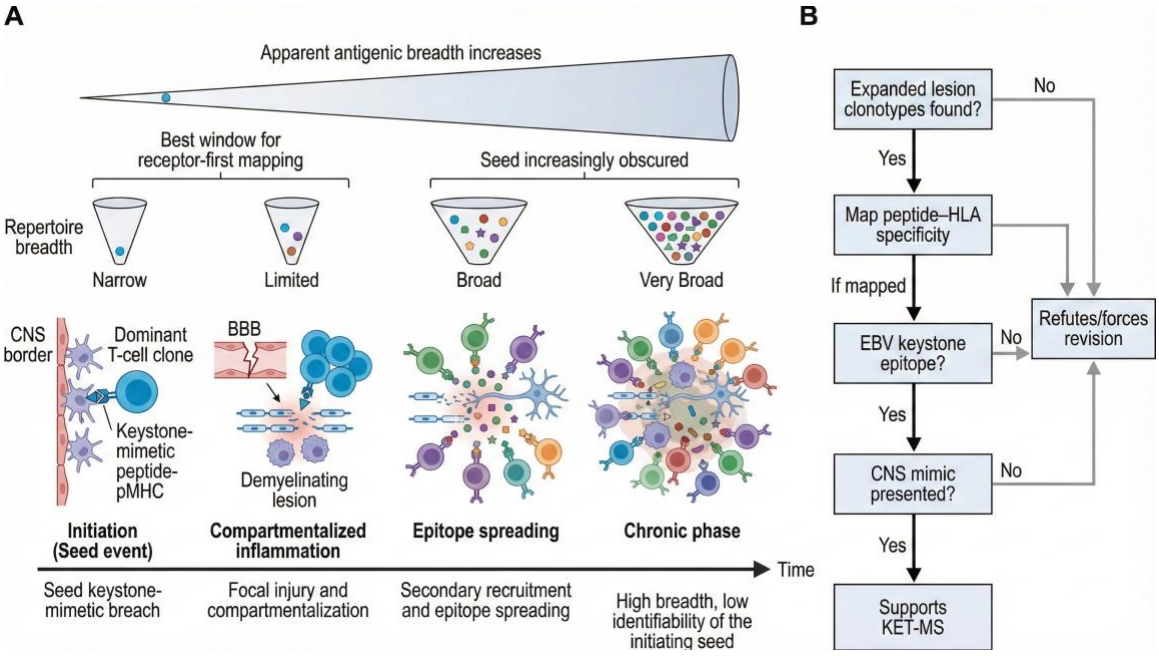

Figure 8. Compartmentalization, epitope spreading, and falsifiability in KET-MS.
(A) Temporal model showing how an initial keystone-mimetic breach yields compartmentalized CNS injury, followed by secondary recruitment of B- and T-cell responses against additional CNS antigens (epitope spreading). As breadth increases, the initiating seed becomes progressively harder to infer retrospectively, motivating early lesion/CSF sampling and receptor-first mapping. **(B)** Falsifiability decision tree defining experimental outcomes that would support or force revision of the KET-MS model.

8.1 Stepwise workflow

1. Define the 'time and site of the crime': obtain active lesion border tissue, when possible, plus paired CSF and blood during early disease or relapse. Phenotype infiltrating T cells and B cells (single-cell RNA, paired-chain TCR/BCR).
2. Prioritize expanded clonotypes: identify clonally expanded TCRs (CD4 cytotoxic and CD8) and CSF-resident BCR lineages. Expansion is the strongest prior for relevance.
3. Capture germline and strain context**:** In parallel with paired-chain TCR recovery, determine host germline TR alleles (genomic typing or AIRR-seq–based allele inference) and, where feasible, EBV strain features. This reduces V-gene misassignment, supports cross-cohort comparison, and enables tests of TR allele × HLA × EBV strain effects on keystone epitope recognition [39 47].
4. Map restricting HLA and epitope: use pMHC-I/II multimer panels, DNA-barcoded peptide–MHC libraries, and functional scanning to identify the cognate peptide(s) for dominant TCR clonotypes. This is the keystone epitope discovery step.
5. Search for mimics only after the epitope is known: (i) scan the EBV proteome and autologous EBV sequence for homologous peptides; (ii) scan the CNS proteome for native

and PTM peptides that recreate the same pMHC surface; (iii) confirm presentation using immunopeptidomics under inflammatory conditions (e.g., IFN-γ exposure).

6. Validate threshold logic: quantify activation thresholds, functional outputs, and requirement for inflammatory context. Demonstrate that mimic density is necessary to cross the inhibitory set point in relevant CNS-resident targets.
7. Close the loop with structure: solve pMHC–TCR (or pMHC–Fab) structures for the keystone and mimic peptides to confirm true geometric mimicry and to avoid 'sequence-level' overinterpretation.

Methodological note: Genome-to-genome and receptor-centric analyses testing HLA, germline TCR, and EBV strain effects must explicitly control for host and viral population structure and shared ancestry. Failure to do so can confound founder effects with true selection or coevolutionary signals and generate spurious associations.

8.2 What would count as a decisive result?

- A recurrent, HLA-stratified keystone epitope recognized by expanded cytotoxic CD4 (or CD8) clonotypes in MS lesions/CSF, with demonstrable cross-recognition of a CNS-presented mimic.
- Demonstration that the CNS mimic is presented in situ (immunopeptidomics or validated surrogate), and that inflammatory context materially lowers the activation threshold.
- Evidence that EBV strain variation (or host modifiers like vitamin D/smoking) shifts the threshold or mimic density required for activation.

9. Translational implications

- Risk prediction: upgrade from HLA-only to HLA plus keystone-epitope-specific receptor signatures (TCR/BCR) and infection-timing markers.
- Prevention: EBV vaccination could, in principle, reduce MS incidence by preventing delayed symptomatic primary infection; KET additionally motivates therapeutic or refocusing approaches in established EBV infection (Section 9.1) [14].
- Targeted immunotherapy: EBV-specific adoptive T-cell approaches have been explored in progressive MS and provide a translational testbed for EBV-targeting logic [72].
- B-cell targeting: the robust clinical effect of B-cell depletion supports a central role for B cells in MS immunopathology [73] [74]. KET interprets this as reducing antigen presentation and keystone-mimic density in the relevant niche.

9.1 Vaccines in a keystone system: why "refocusing" may beat sterilization

Recent evidence that herpes zoster vaccination is associated with lower dementia incidence is a useful "systems-level" precedent for keystone thinking. A Wales-based natural experiment (regression discontinuity around eligibility) estimated that zoster-vaccine eligibility, and the increase in vaccine uptake it produced, was associated with a lower probability of a new

dementia diagnosis over 7 years, with stronger effects in women [75]. Complementary observational and quasi-experimental analyses suggest a similar signal for the recombinant zoster vaccine [76] [77], and a separate analysis across dementia stages suggested potential benefit even after dementia onset [78]. Whatever the eventual mechanistic explanation, these studies reinforce a practical point: intervening in a latent neurotropic herpesvirus can yield downstream neurologic benefit without requiring eradication of the virus from the population. These analyses are observational or quasi-experimental rather than randomized MS-pathogenesis experiments, so residual confounding remains plausible; the value lies in establishing a systems-level precedent that herpesvirus-directed vaccination can alter neurologic outcomes without requiring eradication.

KET frames this as an "immune allocation" problem rather than a binary "infection versus no infection" problem: for persistent, co-evolved organisms, the host often optimizes for regulated equilibrium (containment with minimal tissue cost) rather than maximal sterilization [5]. In animal models, latent herpesvirus infection can even confer heterologous protection against subsequent bacterial challenge, illustrating that lifelong latency can function as more than parasitism in principle [79]. More broadly, the biology of chronic viral infection is increasingly understood as a spectrum of stable host–pathogen states shaped by immune regulation, rather than as simply a failure of clearance [80]. Taken together, the zoster–dementia observations and the chronic-infection literature provide a rational backdrop for caution about sterilizing vaccine goals in a purported keystone system.

When considered in the context of EBV and MS, Keystone Epitope Theory raises a substantive question regarding vaccination strategy. A truly sterilizing EBV vaccine would remove a near-universal, developmentally timed exposure that may contribute to immune calibration. If early EBV exposure is beneficial on average, as proposed by KET, the population-level consequences of eliminating EBV remain uncertain and could include unintended shifts in immune regulation. This is not an argument against EBV vaccination per se, but rather a caution against assuming that prevention of infection is necessarily the optimal endpoint in a co-adapted host–pathogen system.

A more KET-consistent strategy is therefore to prioritize therapeutic or "refocusing" EBV vaccines that aim to (i) reduce the EBV antigenic set-point and follicular amplification, and (ii) reshape immunodominance away from decoy capture and away from any mimic-prone geometries that plausibly sit upstream of CNS injury. Importantly, precedent already exists for a "non-sterilizing but phenotype-modifying" EBV vaccine concept: a recombinant gp350 vaccine reduced infectious mononucleosis in a phase 2 trial yet did not prevent asymptomatic EBV infection, implying that vaccination can alter clinical trajectory without complete sterilization [81]. Contemporary EBV vaccine pipelines also increasingly discuss multi-antigen designs and the need to elicit cellular immunity, reflecting recognition that EBV control is not solved by neutralizing antibody alone [82] [83].

From an MS standpoint, a therapeutic EBV vaccine strategy has pragmatic advantages: it is testable on shorter timelines and can be anchored to mechanistic endpoints aligned with KET. Candidate endpoints include changes in (a) intrathecal B-cell/follicle biology, (b) EBV latency/lytic transcriptional signatures in relevant compartments, (c) expansion/contraction of cytotoxic CD4 and CD8 clonotypes implicated in lesion activity, and (d) the specificity and dynamics of oligoclonal IgG and EBV-directed antibody patterns. The critical design constraint, dictated by KET, is that a "therapeutic refocusing" vaccine must be epitope-aware: if a subset of EBV epitopes (or structurally related mimics) is proximal to tissue injury, indiscriminate boosting of EBV immunity could be counterproductive. Vaccine design in this frame becomes a precision exercise in shifting attention toward constrained control targets while avoiding amplification of the wrong structural program.

Finally, preventive EBV vaccination still deserves attention because EBV contributes to substantial disease burden (including malignancy), and because EBV appears close to necessary for MS in multiple datasets. The strategic claim here is narrower: given uncertainty about EBV's net role in immune coordination and given real-world precedent that herpesvirus vaccination can improve neurologic outcomes without eradication, KET would suggest that therapeutic refocusing and equilibrium management should be favored over population-wide sterilization goals**.** That prediction is falsifiable and should be adjudicated by epitope-resolved immunology and appropriately designed trials.

9.2 Diagnostic opportunities: making MS an epitope-defined disease (at least for a subset)

KET's concrete diagnostic promise is that MS can be endotyped around the **initiating structural program** rather than around late, noisy correlates after epitope spreading. Practically, this suggests the following approaches should be considered:

1. **Lesion-anchored immune forensics**: prioritize sampling "time-and-site-of-crime" T cells (CSF during early relapse; active lesion borders when available; meninges/follicles in progressive disease) and reconstruct expanded clonotypes, then map peptide–HLA specificity forward from those TCRs.
2. **Keystone epitope first, mimic second**: once the key pMHC geometry is defined, search the "sea" of post-translational modification and candidate CNS antigens for structural homologs that could supply the density and context needed for threshold breach.
3. **Risk stratification beyond HLA**: combine HLA risk haplotypes with EBV timing proxies (IM history), EBV serologic fine-specificity (not just seropositivity), and signatures of cytotoxic CD4 programs (including follicle-homing phenotypes) to define a higher-specificity "conditional recall" risk state.
4. **Equilibrium failure readouts**: incorporate longitudinal markers consistent with loss of regulated containment, including compartment-specific reactivation patterns and immune-inhibitory set-point surrogates.

Therapeutic implications of Keystone Epitope Theory in multiple sclerosis

KET reframes therapy as restoring a stable containment regime rather than only suppressing downstream inflammation. It suggests the following therapeutic considerations:

1. **Modulation of intrathecal antigen amplification**
• Target intrathecal and meningeal follicle-like immune structures that sustain chronic antigen presentation and local lymphocyte expansion. This approach is conceptually consistent with the clinical efficacy of B-cell–directed therapies and with evidence for compartmentalized inflammation in progressive multiple sclerosis.

2. **Modulation of EBV-trained effector responses**
• Therapeutic EBV vaccination strategies aimed at altering immunodominance patterns, favoring constrained control targets over decoy or potentially cross-reactive epitopes.
• EBV-specific cellular therapies, or vaccination strategies combined with cellular boosting, as potential approaches to modify follicular immune control in established infection.
• Antigen-specific tolerance strategies, contingent on identification of a disease-relevant mimic axis, including altered peptide ligands or tolerogenic delivery tailored to the relevant antigen-presenting cell context.

3. **Modulation of activation thresholds within CNS-relevant niches**
• If CNS injury reflects a breach of local activation thresholds, interventions that restore inhibitory balance within the relevant niche may be mechanistically important. Caution is warranted, as global checkpoint modulation lacks specificity; the therapeutic objective would be localized restoration of inhibitory control rather than generalized immunosuppression.

10. Limitations and falsifiability

The utility of Keystone Epitope Theory depends on its capacity to generate testable predictions and to be revised or rejected in light of new data. These falsifiability criteria are summarized schematically in Figure 8B. Observations that would materially challenge the KET-MS framework include:

• Absence of consistently expanded T-cell clonotypes in MS lesions or cerebrospinal fluid, or identification of expanded clonotypes that do not map to coherent peptide–MHC specificities.
• Inability to identify EBV-related or keystone-like epitopes among expanded cytotoxic T-cell clonotypes despite high-sensitivity mapping approaches.
• Evidence that candidate CNS mimic peptides are not presented within relevant anatomical niches, or that observed cross-recognition occurs at affinities or antigen densities unlikely to be physiologically meaningful.

11. Conclusion

Multiple sclerosis remains one of the most informative unresolved problems in immunology because it combines a highly reproducible infectious association with marked partial penetrance. EBV exposure is close to universal, yet MS develops in only a small subset of exposed individuals. This discrepancy highlights the need for explanatory frameworks that extend beyond exposure alone and instead address how developmental timing, host genetics, and tissue context interact to shape disease risk.

Within the Keystone Epitope Theory framework, EBV is considered not simply as a trigger but as a long co-adapted persistent infection that may contribute to the organization of immunodominance hierarchies. In this view, EBV-directed immune programs are typically maintained in a regulated equilibrium that supports durable control within follicular B-cell niches. MS becomes biologically plausible when such EBV-trained effector responses are engaged under permissive conditions, including a susceptible HLA background, access to a CNS-relevant anatomical niche, and a breach of local inhibitory thresholds driven by inflammatory context and antigenic mimicry. Once such a breach occurs, compartmentalization of inflammation and epitope spreading are likely to follow and may progressively obscure the initiating antigenic event.

A key implication of this framework is that investigation of MS pathogenesis may benefit from approaches that prioritize immune receptor specificity over cataloguing candidate autoantigens in isolation. A receptor-first strategy, in which expanded lesion or cerebrospinal fluid T-cell clonotypes are identified, their restricting HLA and cognate peptide geometries defined, and only then potential viral or CNS mimics explored, offers a tractable way to constrain the search space. This ordering transforms an otherwise unbounded set of candidate antigens into a finite, experimentally testable workflow and provides a potential route toward defining epitope-based MS endotypes relevant to diagnosis and therapeutic targeting.

Insights from MS have also informed broader aspects of Keystone Epitope Theory. The EBV–MS relationship underscores a general principle in immunology: immune systems are not necessarily optimized for sterilizing all persistent exposures, but rather for maintaining a regulated equilibrium under evolutionary constraints. Pathology may arise when immune allocation, niche access, and inhibitory calibration become misaligned with context. If this principle holds, translational strategies need not default to eliminating persistent organisms; they may instead focus on restoring equilibrium by modulating antigenic amplification, effector targeting, or local activation thresholds.

Finally, the value of Keystone Epitope Theory depends on its capacity to generate testable predictions and to be revised or rejected as new data emerge. Decisive evaluations include whether early lesion-anchored or cerebrospinal fluid–based receptor mapping converges on coherent, HLA-stratified epitope geometries; whether such receptors demonstrably cross-recognize CNS-presented mimics at physiologically plausible densities; and whether targeted modification of the relevant niche or inhibitory set points leads to predictable changes in disease biology. Failure to meet these criteria would necessitate revision of the framework. Conversely, success would substantially advance mechanistic understanding of MS and provide broader insight into how persistent infections shape immunodominance, immune equilibrium, and tissue-specific vulnerability.

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