



Immunity to EBV as revealed by immunodeficiencies

Benjamin Fournier, Sylvain Latour

► To cite this version:

Benjamin Fournier, Sylvain Latour. Immunity to EBV as revealed by immunodeficiencies. Current Opinion in Immunology, 2021, 72, pp.107-115. 10.1016/j.coi.2021.04.003 . hal-03442959

HAL Id: hal-03442959

<https://hal.science/hal-03442959>

Submitted on 23 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

For Current Opinion in Immunology-Host pathogens section

Immunity to EBV as revealed by immunodeficiencies

Benjamin Fournier^{1,2,3} and Sylvain Latour^{1,2}

¹Laboratory of Lymphocyte Activation and Susceptibility to EBV infection, INSERM UMR 1163, Imagine Institute, Paris, France;

²Université de Paris, F75006- Paris, France ;

³Department of Pediatric Immunology, Hematology and Rheumatology, Necker-Enfants Malades, Paris, France

Correspondence to Sylvain Latour, Laboratory of Lymphocyte Activation and Susceptibility to EBV, Equipe Labélisée Ligue contre le Cancer, Inserm UMR 1163, 24 boulevard du Montparnasse, 75015 Paris, France ; Phone : 33 (1) 42 75 43 03 ; FAX : 33 (1) 75 42 21 ; e-mail : sylvain.latour@inserm.fr; ORCID : 0000-0001-8238-4391

Highlights

- Co-stimulatory TNF receptor pathways CD137-CD137L and CD27-CD70 are key for the expansion EBV-specific T cells that control EBV-infected cells.
- Immunity to EBV-infected T/NK and smooth muscle cells involves common but also distinct components to those required for immunity to EBV-infected B cells.
- Pathophysiology of EBV+ T/NK lymphoproliferative disorders depends of complex interplays between germinal and somatic mutations and variations in the EBV genome resulting in immunodeficiency and T/NK cell lymphomagenesis.

Abstract

Epstein-Barr virus infection is the most common viral latent infection in humans and represents one prototypical model to study immunity to viral infections. In that respect, inborn errors of immunity (IEIs) or primary immunodeficiencies (PIDs) predisposing to severe and chronic EBV infections provide peculiar examples to decipher specific molecular and cellular components involved in the immune control of EBV-infected cells. Herein, we discuss the recent knowledge and concepts arising from these studies, with a particular focus on “atypical” EBV infections when EBV enters T, NK and smooth muscle cells, instead of the common “typical” infection of B cells.

Introduction

The Epstein-Barr virus (EBV) or HHV-4 is a double-stranded DNA virus with oncogenic potential belonging to the gamma-1 herpes family [1]. EBV exhibits a unique tropism to humans, in which it establishes for life a latent infection in B lymphocytes, remaining silent in the majority of individuals. Infection by EBV represents one of the most common viral infections in humans since more than 90% of adults are carriers of EBV. During the primary infection, epithelial cells and B lymphocytes in the oropharynx are infected by EBV virions transmitted through saliva [2]. Entry of EBV in B lymphocytes depends of CD21, CD35 and MHC-class II molecules [3]. B lymphocytes are the first and main target of EBV in the initial and persistent stages of the infection [4]. EBV may occasionally enter other cell types including T and NK lymphocytes and smooth muscle cells.

EBV persists as episomes during latency programs and replicates during a lytic program that can both drive non-malignant diseases and malignant tumors (**Table 1**). Those include non-malignant lymphoproliferative disorders, lymphomas of B, T and NK cell origin, gastric and nasopharyngeal carcinomas, smooth muscle tumors (also known as leiomyoma) and virus-induced haemophagocytic lymphohistiocytosis (HLH), an inflammatory disorder arising from dysregulated CD8+T-cell and macrophage expansion and activation. The most frequent subsets remain those of B-cell origin [4].

During the primary infection, EBV replicates in both naive B cells and epithelial cells, the latter amplifying the infection, and induces a strong proliferation of infected B cells. EBV-infected cells that escape from immune response shut down viral gene expression and differentiate into memory B cells becoming invisible to the immune system [5]. Upon re-exposure to their cognate antigen, infected memory B cells could undergo plasma cell

differentiation that reactivates viral lytic production, thereby initiating new infections of neighboring epithelial and B cells [5]. Reactivations of EBV are thought to occur repeatedly during the lifetime [2].

Primary and long-life immune responses to EBV in immunocompetent humans have been largely characterized and mostly involve CD8⁺ T cells, and to a lesser extent CD4⁺ T cells, NK and $\gamma\delta$ -T cells [6–8]. The primary infection is mostly asymptomatic in infants, but when occurred in young adults it can result in infectious mononucleosis (IM), a self-limiting condition usually harmless (but not always). IM is caused by polyclonal expansions of EBV-infected B cells and EBV-specific activated T cells reflecting a particularly robust immune response. Strong expansions of EBV-specific T cells, up to 40% of total blood T cells, are observed in the course of primary infection.

Although EBV infection is benign and asymptomatic in most of the cases, it is considered to be responsible of 1% of cancers worldwide[9]. Furthermore, immunocompromised individuals like patients with AIDS or receiving immunosuppressive treatments are particularly prone to develop EBV-driven B-cell lymphoproliferations/lymphomas and smooth-muscle tumors. Beside acquired immunodeficiencies, there is a group of inborn errors of immunity (IEIs) conferring to carriers a high susceptibility EBV-associated pathologies. These “in natura” models represent unique models to decipher the specific pathways and requirements of immunity to EBV. In the present review we will discuss the recent knowledge and concepts arising from these studies with a particular focus on EBV-driven T, NK and smooth muscle proliferations, for which recent advances have been made in the understanding of their physiopathological mechanisms.

The central role of CD8⁺ T cells in the control of EBV-infected B cells

Up to 400 gene defects causing immunodeficiencies have been reported by now[10]. They can be classified according to the level of predisposition and selectivity to severe and chronic EBV infections they confer, most of them being B-cell lymphoproliferative diseases and HLH. Such an analysis reveals redundant and non-redundant components of immunity toward EBV [11]. Briefly, components of innate immunity including NK cells appear to be dispensable to EBV immunity. Notably, most of SCID patients having received hematopoietic stem cell transplantation or gene therapy did not reconstitute their NK cell compartment and are not prone to chronic EBV infection[12]. The humoral adaptive immunity seems also not be sufficient nor essential to protect against chronic EBV infection. The humoral response to EBV is preserved in most of IEIs associated with a high susceptibility to EBV (see below). IEIs with selective absence of B cells are not informative as affected individuals received Ig replacement treatment and/or are supposed to be resistant to EBV infection (as they have no B cells). However, B-cell deficiencies (e.g. CD40, CD19, CD81) in which B cells are present and still prone to transformation by EBV, are not reportedly associated with severe EBV infections. In contrast, there is much firm evidence that CD8⁺ T-cell responses are key in immunity to EBV. Nonetheless, other T-cell subsets seem not to play a major role. Indeed, defects of CD4⁺ T cells (in HLA-Class II deficiencies), T helper 1 (TH1) (in deficiencies of the IL-12/IFN- γ axis including the recent identified T-bet/TBX21 deficiency [13]), TH17 (in STAT3 deficiency), T follicular helper (in ICOS deficiency), MAIT and NKT cells (in RORC deficiency) are not associated with a susceptibility to develop severe EBV infections [11]. However, CD4⁺ T cells, NK and NKT cells are found to be reduced in several IEIs associated with a high predisposition to EBV infection, suggesting that these

defects could participate to the immunodeficiency, although these cell-subsets appear not to be essential [14].

Specific components of the CD8+ T response required for efficient EBV immunity have been further delineated by identification and studies of IEIs associated with a strong penetrance of severe EBV infections [15], for which >50% patients experienced at least one episode of EBV B-LPD, although they may develop other infections. Those IEIs can be divided in two groups relying on the type of lymphoproliferative disorder and the nature of the mutated gene (**Figure 1**). The first group covers gene defects causing EBV-induced hemophagocytic lymphohistiocytosis (HLH) that impede the granule-dependent cytotoxicity function of NK and CD8+ T cells. Those include mutations in *PRF1*, *RAB27A*, *UNC13D*, *SYT11*, *LYST* and *SH2D1A* [11,16]. The second group is made of IEIs in which EBV infection predominantly drives B-cell lymphoproliferative disorders including B-cell lymphomas. These latter are characterized by impaired activation and expansion of anti-EBV specific T cells resulting from mutations in genes involved in TCR signaling/activation (such as *RASGRP1*, *ITK*, *MAGT1* and *DEF6*), DNA metabolism/synthesis (*CTPS1*) or the co-stimulatory receptors of the TNF-receptor superfamily such as *TNFRSF9* (also known as *CD137/4-1BB*), *CD27* and its ligand *CD70* [17–21]. Within these two groups, mutations in *SH2D1A*, *CD27*, *CD70* and *TNFRSF9* cause a more selective or sometimes exclusive predisposition to EBV-induced immunopathology. This is explained by the specific involvement of these molecules/pathways in direct interactions with EBV-infected B cells. *SH2D1A* (also known as *SAP*) is an intracellular signaling molecule required for the function of the SLAM family receptors (SLAM-R), the most important SLAM-R involved in the control of EBV infection being 2B4 that triggers NK and T cell-cytotoxicity towards EBV-infected B cells. Upon EBV infection, B cells express high levels of CD48, the

ligand of 2B4 [11,22]. Similarly, CD27 and CD137 sustain T-cell expansion and terminal differentiation of EBV-reactive T-cells through interactions with their ligands (CD70 and CD137L) that accumulate on B cell surface when infected by EBV. In MAGT1 deficiency, interestingly, the susceptibility to EBV infection is associated with a defect in the expression of NKG2D, a receptor involved in cytotoxic responses of NK and CD8+ T cells [19]. Recent observations suggest that MAGT1 deficiency also causes impaired expression of CD70 and CD28 [23]. These studies highlighted that the T-cell response to EBV-infected B cells comprises two critical steps, the expansion and the effector phases, which when impeded result in two distinct lymphoproliferative disorders, HLH and B-LPDs respectively (**Figure 2**).

Physiopathology and immune control of EBV+ T/NK LPD

EBV+ T/NK LPD are very rare and aggressive diseases compared to their B cell counterparts, in which EBV infects CD8+, CD4+, $\gamma\delta$ -T and/or NK cells. EBV+ T/NK LPD have been restricted to Asian and Central/South American populations[24,25]. However, we recently showed that T/NK LPD can also occur in Caucasian and African individuals indicating they are likely underdiagnosed in Western countries[26]. EBV+ T/NK LPDs encompass overtly malignant diseases that include extra-nodal NK/T cell lymphoma (ENKTL), aggressive NK cell leukemia (ANKL, mostly found in adults), systemic EBV+ T cell lymphoma of childhood (STCL mostly found in childhood) and a non-malignant lymphoproliferative disorder, the T/NK-cell chronic active EBV (also termed as “CAEBV”) divided into a systemic and two cutaneous forms (hydroa vacciniforme-like lymphoproliferation (HVL) and severe mosquito bite allergy(SMBA)) (**Table 1**) [27]. Boundaries between these entities are permeable and they can represent sometimes different states of a same

disease. In that respect, CAEBV may evolve suddenly and rapidly towards malignant lesions or HLH. They are characterized by high and/or persistent (over years for CAEBV) blood EBV loads, not or poorly relieved by B-cell depletion. Until recently, definitive diagnosis was established by histologically evidence of EBV+ T- and/or NK cells in tissue biopsies. The recent development of easy and reliable flow-fish assays for Epstein–Barr virus-encoded small RNAs (EBERs) from blood samples, allowing identification of EBV-infected cell subsets, should now increase the diagnosis of NK/T LPDs and facilitate their studies[26,28].

The etiology of EBV-driven T/NK LPDs is not well understood and their occurrence likely depends of the convergence of multiple factors and complex mechanisms explaining their rarity. Specific strains of EBV, heterogeneous germline genetic factors (including IEI), somatic mutations and immune defects are suspected to be causal determinants. Persistence for years of EBV+ T/NK cells in many cases suggests that T/NK cells represent an alternative and additional reservoir for EBV and indicates that the immune system is at fault. Along these lines, anti-EBV CD8+ T cells have been found to be significantly lower in some patients with CAEBV [29], and myeloid-derived suppressor cells (MDSC) were recently found to accumulate in some CAEBV patients [29]. However, EBV+ T/NK cells are rarely found in the context primary and secondary immune deficiencies [30], albeit one study repeatedly identified EBV+ T cells from HIV+ children[31]. Only rare cases of EBV+ T/NK LPD have been reported in IEIs, including concomitant EBV infection of B, T and smooth muscle cells in a patient carrying of a hypomorphic *IL2RG* hemizygous mutation [32], HV-like LPD in a patient with plausible *GATA2* haploinsufficiency [33], NK-cell CAEBV in a *FANCA* deficient patient[34], T-cell CAEBV in a patient with ICF2

[35] and systemic EBV+ T cell lymphoma of childhood associated with a 22q11.2del syndrome [36].

Recently several studies shed new light on pathological and immune mechanisms involved in T/NK LPD (**Figure 3**). Okuno et al. analyzed 77 Japanese patients with systemic CAEBV and found that a substantial part of patients carried in their infected T or NK cells potential driver somatic mutations in pathways (JAK-STAT, ERK-MAPK and the RNA helicase DDX3X) similar to those detected in malignant T/NK LPD, the most common mutated gene being *DDX3X* [37]. These mutations accumulate in clones of infected cells over time and may lead to overt T/NK lymphoma or HLH, thus supporting that CAEBV is a premalignant condition. The large majority of patients were carriers of the EBV type 1 strain as shown previously[38–40], thus indicating that the EBV type 2 strain cannot selectively account for NK/T LPDs as it was proposed[41]. Interestingly, thirty-five percent of EBV genomes displayed intragenic deletions in a single lymphoid clone and were concomitantly associated with potential driver somatic mutations. These deletions predominantly include BART microRNA clusters and/or genes involved in viral particle production, which both are involved in the expression of lytic transcripts. These lesions may result in a “lytic abortive infection” associated with increased expression of lytic transcripts without virion production and have been proposed to bear potential oncogenic properties[42]. Finally, a last important contribution of Okuno et al. is the observation that in the rare patients exhibiting both infected T and NK subsets, both shared same somatic mutations and EBV genome variations, thus strongly favoring the hypothesis of the infection of a common T/NK lymphoid precursor rather than infection of differentiated cells. As no EBV+ CD34+ cell was detected in patients [43], early thymic precursors that are known to express CD21 represent good candidates [44,45] and may represent

one way for EBV entry in T/NK cells, whose infectious mechanism(s) remains debated (as differentiated T and NK cells are not expressing CD21).

Rodriguez et al., reported the identification of familial form of systemic CAEBV in two siblings caused by a homozygous loss-of-function (LOF) mutation in *TNFRSF9* [46]. One sibling was symptomatic and died of CD8+ T lymphoma, while the other was asymptomatic but presented high EBV loads and circulating EBV-infected T cells that persisted for years. Interestingly, the symptomatic sibling was also carrier of a second genetic event, a homozygous LOF mutation in *PIK3CD* that was found to increase T-cell proliferation. Thus, *PIK3CD* deficiency may provide a cellular and genetic context supporting the persistence of EBV-infected T/NK and thus fostering accumulation of somatic mutations. These observations strengthen the hypothesis that T/NK LPDs have an oligogenic or polygenic inheritance. Furthermore, they also indicate that pathways controlling EBV-infected B cells may, to a certain extent, be also involved in the surveillance of EBV-infected T/NK cells, as *TNFRSF9* (CD137/4-1BB) deficiency also predisposes to EBV-driven B-LPDs (see below). Confirming the key role of the CD137-CD137L pathway in immune control of EBV-infected T cells, we recently identified a patient with a CD137L deficiency caused by a homozygous mutation who developed a broad susceptibility to EBV with the presence of EBV-infected T, B and smooth muscle cells (B. Fournier and S. Latour al., unpublished data). So far, no CD137L expression was detected on EBV-infected T- or NK-cells, which may be expressed early in the infection and down-regulated thereafter. However, some hematological malignancies including T-cell lymphomas expressed high levels of CD137L [47,48]. Other cell types may express CD137L and indirectly enhance immunity against infected cells, such as antigen-presenting cells and endothelial cells in the tumor microenvironment [49,50].

More recently, confirmation of familial forms of T/NK LPD was provided by the identification of three siblings with CD16A deficiency caused by a bi-allelic deletion/recombination in *FCGR3A* coding CD16A [51], a low affinity receptor for the Fc portion of IgG expressed on NK cells, monocytes and granulocytes. CD16A mediates antibody-dependent cellular responses including NK-cell cytotoxicity. One sibling developed a symptomatic systemic CAEBV, while the two other siblings had only elevated EBV without any clinical manifestations. This could indicate the implication of additional genetic factors (not present in the two asymptomatic siblings) that triggered the disease in the symptomatic sibling, as found in the symptomatic patient with TNFRSF9 deficiency who had also a PIK3CD deficiency. Interestingly, this study suggests that components of innate immunity, considered to be dispensable in the immune control to EBV-infected B cells, are required for the immunity to EBV-infected NK/T-cells.

Immunity to EBV+ smooth muscle cells

EBV+ smooth muscle tumors (EBV+SMT) are very rare tumors that were first identified in immunosuppressed patients after organ transplantation [52] and in AIDS patients [53], however, EBV+ SMT remain very rare even in this context. Analysis of EBV genomes revealed that multi-centric EBV+SMT (with multiple organ locations) arise from multiple clonal infections rather than metastasis [53]. As for T and NK cells, mechanisms leading to EBV entry in smooth muscle cells that do not express CD21, are unknown. EBV+SMT are also very rare in the setting of IEIs. Single cases of have been reported in SCIDs caused by mutations in *ZAP70* or *IL2RG*, GATA2-deficiency and Ataxia-telangiectasia [54]. In RASGRP1 deficiency and ITK deficiency that predispose to EBV, two cases and one case have been documented, respectively [17,55,56].

Finally, the patient with CD137L deficiency we recently identified presented with multiple EBV+ smooth tumors in different organs (B. Fournier and S. Latour al., unpublished data). More interesting is the CARMIL2 deficiency; most of the patients had a mild positive blood EBV PCR [57] and several developed EBV+ SMT [58,59], which makes RLTPR/CARMIL2-deficiency, the disease with the highest incidence of EBV+ SMT. Notably, none of RLTPR-deficient patients developed symptomatic EBV+ B-LPD or EBV+ T/NK LPD. RLTPR is a large cytosolic adaptor/scaffolding protein that bridges the CD28 T-cell costimulatory molecule to the NF- κ B pathway [60], however, it is very likely that RLTPR is also involved in other pathways [57,58]. When RLTPR is defective, T-cell responses to CD28 stimulation are impeded [57,58]. Importantly, RLTPR-deficient T cells failed to control autologous EBV-transformed cell lines in vitro which is indicative of an abnormal immune response to EBV. Thus, CD137 and CD28 T-cell co-stimulation pathways may be particularly important in the immune response to EBV-infected smooth muscle cells, but likely indirectly through other cell types as smooth muscle cells do not express CD137L and CD80/CD86 (the ligands of CD28). Other RLTPR-dependent pathways than CD28 might also be involved. Given the rarity of EBV+SMT, it is difficult to draw any solid conclusion, but the four cases in IELs predisposing to EBV+ B-cell LPD (RASGRP1, ITK, CD137L) may suggest that the same pathways are at work to control EBV-infected smooth cells and/or that defective immune control of EBV-infected B cells could facilitate the spread of EBV infection to other cell types. However, RLTPR appears to be a critical and selective molecule for the immune control of EBV+SMT (since RLTPR-deficient patients did not present symptomatic EBV+ B/T/NK-LPD).

Conclusion

The recent progress in the characterization of IELs predisposing to EBV infection has highlighted the importance of both expansion and effector steps of the CD8⁺ T-cell responses during EBV infection, in particular the critical role of co-stimulatory pathways dependent of the TNF receptors CD27 and CD137. Studies of “atypical” EBV infections causing NK/T and SMT-associated-cell proliferative pathologies suggest common, but also distinct immune components at play, as well as divergent pathological mechanisms than those involved in “typical” EBV-driven B-cell pathologies. Defects in adaptive immunity in addition to cancer driver somatic or germinal mutations may be important requirements for the establishment of EBV⁺ T/NK LPD (in contrast cancer driver mutations may be less necessary for EBV⁺ B-LPD). RLTPR-dependent pathways appear to be critical for the immune control of EBV⁺ SMT. Identifications of additional inherited genetic susceptibilities to EBV in the next future, in particular those associated with EBV⁺ NK/T LPD and EBV⁺ SMT will increase our knowledge and understanding of these very rare diseases often neglected.

Acknowledgements

We thank all the team members for their contribution. S. Latour is a senior scientist at the Centre National de la Recherche Scientifique (France). B.F. was supported by the Fondation de la Recherche Médicale (France). The laboratory of S.L. receives fundings and supports from the Ligue Contre le Cancer-Equipe Labelisée (France), Institut National de la Santé et de la Recherche Médicale (France), the French Foundation of Rare Diseases (France), the Agence Nationale de Recherche ANR (France) (ANR-14-CE14-0028-01, ANR-18-CE15-0025-01 to S.L. and ANR-10-IAHU-01 to Institut Imagine), the Institut National du Cancer (France) (INCa-PEDIAC-2020, PLBIO20-121), the Société

Française de Lutte PLBIO2020 contre les Cancers et Leucémies de l'Enfant et de l'Adolescent, AREMIG (France), and the Fédération Enfants et Santé (France).

References

1. Young LS, Yap LF, Murray PG: **Epstein-Barr virus: more than 50 years old and still providing surprises.** *Nat Rev Cancer* 2016, **16**:789–802.
2. Thorley-Lawson DA: **EBV Persistence-Introducing the Virus.** *Curr Top Microbiol Immunol* 2015, **390**:151–209.
3. Connolly SA, Jardetzky TS, Longnecker R: **The structural basis of herpesvirus entry.** *Nature Reviews Microbiology* 2021, **19**:110–121.
4. Shannon-Lowe C, Rickinson A: **The Global Landscape of EBV-Associated Tumors.** *Front Oncol* 2019, **9**.
5. Chiu Y-F, Sugden B: **Epstein-Barr Virus: The Path from Latent to Productive Infection.** *Annu Rev Virol* 2016, **3**:359–372.
6. Long HM, Meckiff BJ, Taylor GS: **The T-cell Response to Epstein-Barr Virus-New Tricks From an Old Dog.** *Front Immunol* 2019, **10**:2193.
7. Djaoud Z, Guethlein LA, Horowitz A, Azzi T, Nemat-Gorgani N, Olive D, Nadal D, Norman PJ, Münz C, Parham P: **Two alternate strategies for innate immunity to Epstein-Barr virus: One using NK cells and the other NK cells and $\gamma\delta$ T cells.** *Journal of Experimental Medicine* 2017, **214**:1827–1841.
8. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD: **The immunology of Epstein-Barr virus-induced disease.** *Annu Rev Immunol* 2015, **33**:787–821.
9. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM: **Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis.** *The Lancet Global Health* 2020, **8**:e180–e190.
10. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, Franco JL, Holland SM, Klein C, Morio T, et al.: **Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee.** *J Clin Immunol* 2020, **40**:24–64.
11. Latour S, Fischer A: **Signaling pathways involved in the T-cell-mediated immunity against Epstein-Barr virus: Lessons from genetic diseases.** *Immunological Reviews* 2019, **291**:174–189.
12. Vély F, Barlogis V, Vallentin B, Neven B, Piperoglou C, Ebbo M, Perchet T, Petit M, Yessaad N, Touzot F, et al.: **Evidence of innate lymphoid cell redundancy in humans.** *Nature Immunology* 2016, **17**:1291–1299.

13. Yang R, Mele F, Worley L, Langlais D, Rosain J, Benhsaien I, Elarabi H, Croft CA, Doisne J-M, Zhang P, et al.: **Human T-bet Governs Innate and Innate-like Adaptive IFN- γ Immunity against Mycobacteria.** *Cell* 2020, **183**:1826-1847.e31.
14. Latour S, Winter S: **Inherited Immunodeficiencies With High Predisposition to Epstein–Barr Virus-Driven Lymphoproliferative Diseases.** *Front Immunol* 2018, **9**.
15. Tangye SG, Latour S: **Primary immunodeficiencies reveal the molecular requirements for effective host defense against EBV infection.** *Blood* 2020, **135**:644–655.
16. Sepulveda FE, de Saint Basile G: **Hemophagocytic syndrome: primary forms and predisposing conditions.** *Curr Opin Immunol* 2017, **49**:20–26.
17. Winter S, Martin E, Boutboul D, Lenoir C, Boudjemaa S, Petit A, Picard C, Fischer A, Leverger G, Latour S: **Loss of RASGRP1 in humans impairs T-cell expansion leading to Epstein-Barr virus susceptibility.** *EMBO Molecular Medicine* 2018, **10**:188–199.
18. Izawa K, Martin E, Soudais C, Bruneau J, Boutboul D, Rodriguez R, Lenoir C, Hislop AD, Besson C, Touzot F, et al.: **Inherited CD70 deficiency in humans reveals a critical role for the CD70–CD27 pathway in immunity to Epstein-Barr virus infection.** *Journal of Experimental Medicine* 2017, **214**:73–89.
19. Chaigne-Delalande B, Li F-Y, O'Connor GM, Lukacs MJ, Jiang P, Zheng L, Shatzer A, Biancalana M, Pittaluga S, Matthews HF, et al.: **Mg²⁺ Regulates Cytotoxic Functions of NK and CD8 T Cells in Chronic EBV Infection Through NKG2D.** *Science* 2013, **341**:186–191.
20. Martin E, Minet N, Bosch A-C, Sanquer S, Sobrino S, Lenoir C, Villartay JP de, Leite-de-Moraes M, Picard C, Soudais C, et al.: **Impaired lymphocyte function and differentiation in CTPS1-deficient patients result from a hypomorphic homozygous mutation.** *JCI Insight* 2020, **5**.
21. Fournier B, Tusseau M, Villard M, Malcus C, Chopin E, Martin E, Cordeiro DJ, Fabien N, Fusaro M, Gauthier A, et al.: **DEF6 deficiency, a mendelian susceptibility to EBV infection, lymphoma and autoimmunity.** *J Allergy Clin Immunol* 2020, doi:10.1016/j.jaci.2020.05.052.
22. Tangye SG: **XLP: Clinical Features and Molecular Etiology due to Mutations in SH2D1A Encoding SAP.** *J Clin Immunol* 2014, **34**:772–779.
- *23. Matsuda-Lennikov M, Biancalana M, Zou J, Ravell JC, Zheng L, Kanellopoulou C, Jiang P, Notarangelo G, Jing H, Masutani E, et al.: **Magnesium transporter 1 (MAGT1) deficiency causes selective defects in N-linked glycosylation and expression of immune-response genes.** *J Biol Chem* 2019, **294**:13638–13656.

This paper demonstrated that MAGT1 is critical for proper N-glycosylation required for expression and function of several immune and non-immune proteins including NKG2D, CD70 and CD28.

24. Kimura H, Ito Y, Kawabe S, Gotoh K, Takahashi Y, Kojima S, Naoe T, Esaki S, Kikuta A, Sawada A, et al.: **EBV-associated T/NK-cell lymphoproliferative diseases in nonimmunocompromised hosts: prospective analysis of 108 cases.** *Blood* 2012, **119**:673–686.
25. Kawamoto K, Miyoshi H, Suzuki T, Kozai Y, Kato K, Miyahara M, Yujiri T, Choi I, Fujimaki K, Muta T, et al.: **A distinct subtype of Epstein-Barr virus-positive T/NK-cell lymphoproliferative disorder: adult patients with chronic active Epstein-Barr virus infection-like features.** *Haematologica* 2018, **103**:1018–1028.
- **26. Fournier B, Boutboul D, Bruneau J, Miot C, Boulanger C, Malphettes M, Pellier I, Dunogu   B, Terrier B, Suarez F, et al.: **Rapid identification and characterization of infected cells in blood during chronic active Epstein-Barr virus infection.** *J Exp Med* 2020, **217**.

This study reported a reliable and easy flow-FISH cytometry assay that detects cells expressing EBV-encoded small RNAs (EBERs), allowing rapid identification of infected T, NK and B cells from blood. A cohort including patients of caucasian and african origins with EBV+T/NK LPD was characterized by this mean. The authors also showed that this method represents a powerful tool to study the pathophysiological mechanisms of EBV+T/NK LPD.

27. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD, et al.: **The 2016 revision of the World Health Organization classification of lymphoid neoplasms.** *Blood* 2016, **127**:2375–2390.
- **28. Collins P, Fox CP, George LC, Pearce H, Ryan GB, De Santo C, Mussai FJ, Lewis D, Long HM, Shannon-Lowe CD: **Characterising EBV-associated lymphoproliferative diseases and the role of myeloid-derived suppressor cells.** *Blood* [date unknown], doi:10.1182/blood.2020005611.

This paper uses a flow-FISH cytometry assay (similar as in ref 16) to detect EBER+/EBV+ cells to analyze patients with EBV+ NK/T LPD. They observed in almost every CAEBV patients tested large numbers of myeloid derived suppressor cells that may participate to the host's failure to control the growth of EBV+ T/NK cells.

29. Sugaya N, Kimura H, Hara S, Hoshino Y, Kojima S, Morishima T, Tsurumi T, Kuzushima K: **Quantitative Analysis of Epstein-Barr Virus (EBV)–Specific CD8+ T Cells in Patients with Chronic Active EBV Infection.** *J Infect Dis* 2004, **190**:985–988.
30. Kuno M, Ito A, Maeshima AM, Taniguchi H, Tanaka T, Inamoto Y, Kurosawa S, Kim S-W, Fukuda T: **T-cell posttransplant lymphoproliferative disorders after allogeneic hematopoietic cell transplantation.** *Int J Hematol* 2020, doi:10.1007/s12185-020-02890-1.
31. Bekker V, Scherpbier H, Beld M, Piriou E, Breda A van, Lange J, Leth F van, Jurriaans S, Alders S, Dillen PW, et al.: **Epstein-Barr Virus Infects B and Non-B Lymphocytes in HIV-1–Infected Children and Adolescents.** *J Infect Dis* 2006, **194**:1323–1330.

32. Tanita K, Hoshino A, Imadome K-I, Kamiya T, Inoue K, Okano T, Yeh T, Yanagimachi M, Shiraishi A, Ishimura M, et al.: **Epstein-Barr Virus-Associated $\gamma\delta$ T-Cell Lymphoproliferative Disorder Associated With Hypomorphic IL2RG Mutation**. *Front Pediatr* 2019, **7**.
33. Cohen JL, Dropulic L, Hsu AP, Zerbe CS, Krogmann T, Dowdell K, Hornung RL, Lovell J, Hardy N, Hickstein D, et al.: **Association of GATA2 Deficiency With Severe Primary Epstein-Barr Virus (EBV) Infection and EBV-associated Cancers**. *Clin Infect Dis* 2016, **63**:41–47.
34. Sekinaka Y, Mitsuiki N, Imai K, Yabe M, Yabe H, Mitsui-Sekinaka K, Honma K, Takagi M, Arai A, Yoshida K, et al.: **Common Variable Immunodeficiency Caused by FANC Mutations**. *J Clin Immunol* 2017, **37**:434–444.
35. Kamae C, Imai K, Kato T, Okano T, Honma K, Nakagawa N, Yeh T-W, Noguchi E, Ohara A, Shigemura T, et al.: **Clinical and Immunological Characterization of ICF Syndrome in Japan**. *J Clin Immunol* 2018, **38**:927–937.
36. Itoh S, Ohno T, Kakizaki S, Ichinohasama R: **Epstein-Barr virus–positive T-cell lymphoma cells having chromosome 22q11.2 deletion: an autopsy report of DiGeorge syndrome**. *Human Pathology* 2011, **42**:2037–2041.
- **37. Okuno Y, Murata T, Sato Y, Muramatsu H, Ito Y, Watanabe T, Okuno T, Murakami N, Yoshida K, Sawada A, et al.: **Defective Epstein–Barr virus in chronic active infection and haematological malignancy**. *Nature Microbiology* 2019, doi:10.1038/s41564-018-0334-0.

In this paper, the authors showed that EBV+ T/NK cells originate from an EBV-infected lymphoid progenitor that acquires driver cancer somatic mutations (the most common being in *DDXD3*), causing clonal evolution in multiple cell lineages. They also found that EBV genome harboured frequent intragenic deletions suggesting a unique role of these lesions in neoplastic proliferation of EBV-infected T/NK cells.

38. Quintanilla-Martinez L, Kumar S, Fend F, Reyes E, Teruya-Feldstein J, Kingma DW, Sorbara L, Raffeld M, Straus SE, Jaffe ES: **Fulminant EBV+ T-cell lymphoproliferative disorder following acute/chronic EBV infection: a distinct clinicopathologic syndrome**. *Blood* 2000, **96**:443–451.
39. Cohen JL, Manoli I, Dowdell K, Krogmann TA, Tamura D, Radecki P, Bu W, Turk S-P, Liepshutz K, Hornung RL, et al.: **Hydroa vacciniforme–like lymphoproliferative disorder: an EBV disease with a low risk of systemic illness in whites**. *Blood* 2019, **133**:2753–2764.
40. Imajoh M, Hashida Y, Murakami M, Maeda A, Sato T, Fujieda M, Wakiguchi H, Daibata M: **Characterization of Epstein–Barr virus (EBV) BZLF1 gene promoter variants and comparison of cellular gene expression profiles in Japanese patients with infectious mononucleosis, chronic active EBV infection, and EBV-associated hemophagocytic lymphohistiocytosis**. *J Med Virol* 2012, **84**:940–946.

41. Coleman CB, Lang J, Sweet LA, Smith NA, Freed BM, Pan Z, Haverkos B, Pelanda R, Rochford R: **Epstein-Barr Virus Type 2 Infects T Cells and Induces B Cell Lymphomagenesis in Humanized Mice.** *Journal of Virology* 2018, **92**:e00813-18.
42. Münz C: **Latency and lytic replication in Epstein–Barr virus-associated oncogenesis.** *Nature Reviews Microbiology* 2019, **17**:691–700.
43. Ohga S, Ishimura M, Yoshimoto G, Miyamoto T, Takada H, Tanaka T, Ohshima K, Ogawa Y, Imadome K-I, Abe Y, et al.: **Clonal origin of Epstein-Barr virus (EBV)-infected T/NK-cell subpopulations in EBV-positive T/NK-cell lymphoproliferative disorders of childhood.** *J Clin Virol* 2011, **51**:31–37.
44. Smith NA, Coleman CB, Gewurz BE, Rochford R: **CD21 (Complement Receptor 2) Is the Receptor for Epstein-Barr Virus Entry into T Cells.** *Journal of Virology* 2020, **94**.
45. Pekalski ML, García AR, Ferreira RC, Rainbow DB, Smyth DJ, Mashar M, Brady J, Savinykh N, Dopico XC, Mahmood S, et al.: **Neonatal and adult recent thymic emigrants produce IL-8 and express complement receptors CR1 and CR2.** *JCI Insight* 2017, **2**.
- **46. Rodriguez R, Fournier B, Cordeiro DJ, Winter S, Izawa K, Martin E, Boutboul D, Lenoir C, Fraitag S, Kracker S, et al.: **Concomitant PIK3CD and TNFRSF9 deficiencies cause chronic active Epstein-Barr virus infection of T cells.** *Journal of Experimental Medicine* 2019, doi:10.1084/jem.20190678.

In this paper, the authors identified bi-allelic loss-of-function mutations in *PIK3CD* and *TNFRSF9* (CD137/4-1BB) in a patient who developed a fatal CAEBV. They showed that *PIK3CD* deficient T cells exhibited enhanced proliferation that could sustain EBV+ T cells to persist and accumulate, while on the other hand, CD137 deficiency may explain the host's failure to contain growth of EBV+ T cells, highlighting the critical role of the CD137/CD137L pathway in immunity to EBV.

47. Kamijo H, Miyagaki T, Shishido-Takahashi N, Nakajima R, Oka T, Suga H, Sugaya M, Sato S: **Aberrant CD137 ligand expression induced by GATA6 overexpression promotes tumor progression in cutaneous T-cell lymphoma.** *Blood* 2018, **132**:1922–1935.
48. Zhao S, Zhang H, Xing Y, Natkunam Y: **CD137 ligand is expressed in primary and secondary lymphoid follicles and in B-cell lymphomas: diagnostic and therapeutic implications.** *Am J Surg Pathol* 2013, **37**:250–258.
49. Seko Y, Sugishita K, Sato O, Takagi A, Tada Y, Matsuo H, Yagita H, Okumura K, Nagai R: **Expression of Costimulatory Molecules (4-1BBL and Fas) and Major Histocompatibility Class I Chain-Related A (MICA) in Aortic Tissue with Takayasu's Arteritis.** *JVR* 2004, **41**:84–90.
50. Olofsson Peder S., Söderström Leif Å., Wågsäter Dick, Sheikine Yuri, Ocaya Pauline, Lang François, Rabu Catherine, Chen Lieping, Rudling Mats, Aukrust Pål, et al.: **CD137 Is Expressed in Human Atherosclerosis and Promotes Development of Plaque Inflammation in Hypercholesterolemic Mice.** *Circulation* 2008, **117**:1292–1301.

- *51. Pérez-Portilla A, Moraru M, Blázquez-Moreno A, Kolb P, García-Morato MB, Ranganath T, Estes G, Gianelli C, Rodríguez-Pena R, Lozano-Rodríguez R, et al.: **Identification of the first cases of complete CD16A deficiency: Association with persistent EBV infection.** *Journal of Allergy and Clinical Immunology* 2020, **145**:1288–1292.

In this paper, the authors identified the first complete CD16A deficiency in a patient with a CAEBV and two asymptomatic siblings. CD16A-deficient individuals exhibited decreased numbers of immature NK cells and CD16A-deficient NK cells displayed defective antibody-mediated cell cytotoxicity.

52. Jossen J, Chu J, Hotchkiss H, Wistinghausen B, Iyer K, Magid M, Kamath A, Roayaie S, Arnon R: **Epstein–Barr virus-associated smooth muscle tumors in children following solid organ transplantation: A review.** *Pediatric Transplantation* 2015, **19**:235–243.
53. Purgina B, Rao UNM, Miettinen M, Pantanowitz L: **AIDS-Related EBV-Associated Smooth Muscle Tumors: A Review of 64 Published Cases.** *Pathology Research International* 2011, **2011**:e561548.
54. Magg T, Schober T, Walz C, Ley-Zaporozhan J, Facchetti F, Klein C, Hauck F: **Epstein–Barr Virus+ Smooth Muscle Tumors as Manifestation of Primary Immunodeficiency Disorders.** *Front Immunol* 2018, **9**.
55. Serwas NK, Cagdas D, Ban SA, Bienemann K, Salzer E, Tezcan İ, Borkhardt A, Sanal O, Boztug K: **Identification of ITK deficiency as a novel genetic cause of idiopathic CD4+ T-cell lymphopenia.** *Blood* 2014, **124**:655–657.
56. Somekh I, Marquardt B, Liu Y, Rohlf M, Hollizeck S, Karakukcu M, Unal E, Yilmaz E, Patisroglu T, Cansever M, et al.: **Novel Mutations in RASGRP1 are Associated with Immunodeficiency, Immune Dysregulation, and EBV-Induced Lymphoma.** *J Clin Immunol* 2018, **38**:699–710.
- *57. Wang Y, Ma CS, Ling Y, Bousfiha A, Camcioglu Y, Jacquot S, Payne K, Crestani E, Roncagalli R, Belkadi A, et al.: **Dual T cell– and B cell–intrinsic deficiency in humans with biallelic RLTPR mutations.** *Journal of Experimental Medicine* 2016, **213**:2413–2435.
- *58. Schober T, Magg T, Laschinger M, Rohlf M, Linhares ND, Puchalka J, Weisser T, Fehlner K, Mautner J, Walz C, et al.: **A human immunodeficiency syndrome caused by mutations in CARMIL2.** *Nature Communications* 2017, **8**:14209.
- *59. Yonkof JR, Gupta A, Rueda CM, Mangray S, Prince BT, Rangarajan HG, Alshahrani M, Varga E, Cripe TP, Abraham RS: **A Novel Pathogenic Variant in CARMIL2 (RLTPR) Causing CARMIL2 Deficiency and EBV-Associated Smooth Muscle Tumors.** *Front Immunol* 2020, **11**.

These three papers reported a new combined immunodeficiency caused by bi-allelic mutations in *RLTPR/CARMIL2*. CD28-dependent T cell responses were impaired in *RLTPR/CARMIL2*-deficient patients. Some patients had persistent EBV viremia and several developed EBV+ smooth muscle tumors.

60. Roncagalli R, Cucchetti M, Jarmuzynski N, Grégoire C, Bergot E, Audebert S, Baudalet E, Menoita MG, Joachim A, Durand S, et al.: **The scaffolding function of the RLTPR protein explains its essential role for CD28 co-stimulation in mouse and human T cells.** *Journal of Experimental Medicine* 2016, **213**:2437–2457.

Figures

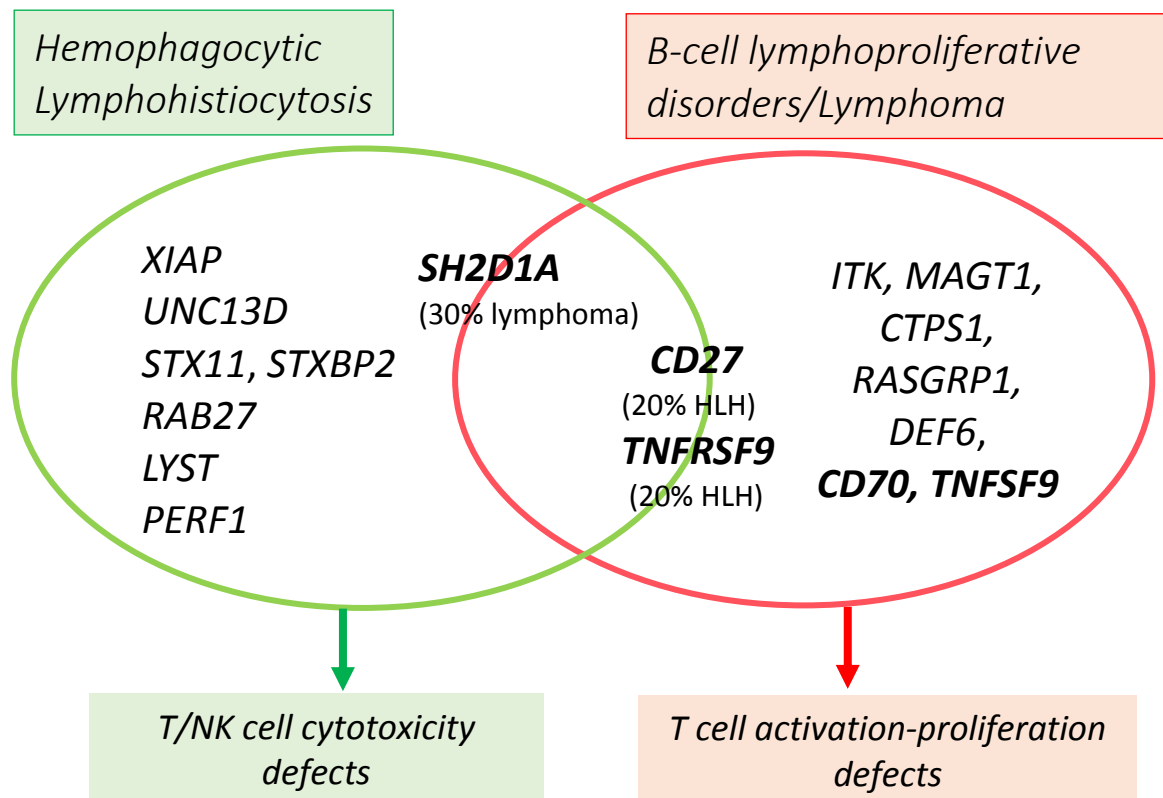


Figure 1. Classification of gene defects predisposing to EBV driven HLH or B-cell lymphoproliferative disorders. In the red circle are shown gene defects associated with impaired cell-cytotoxicity causing HLH, while in the green circle here are the gene defects associated with impaired T-cell activation/expansion causing B-LPD/lymphoma. In bold, gene defects with a highly selective predisposition to EBV. In brackets, % of patients having developed HLH or B lymphoma for defects associated with both HLH and B-LPD/lymphoma.

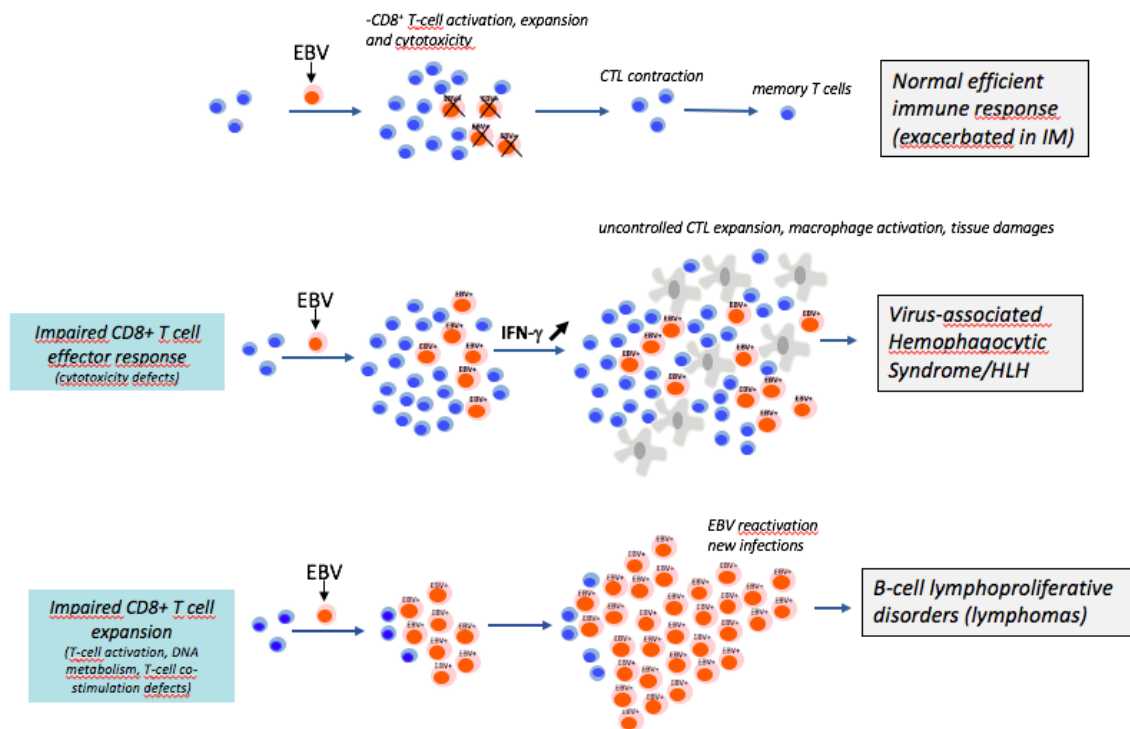


Figure 2. Defects in T-cell expansion and effector phases in the pathophysiology of EBV-driven diseases. Normal immune response to EBV requires a robust expansion of EBV-specific T cells (expansion phase) to counteract and efficiently eliminate EBV-infected proliferating B cells through the effector cytotoxic response of CD8⁺ T cells (effector phase). The activated T-cell pool then contracts and specific memory T cells come up. Gene defects causing impaired T-cell cytotoxicity activity/killing result in the persistence of EBV-infected cells that are not eliminated. Persistence of infected cells sustains uncontrolled activation and expansion of T cells that cannot kill infected cells, but can still proliferate and produce large amounts of pro-inflammatory cytokines including IFN- γ . In that setting, macrophages are activated (by cytokines released by activated T cells) causing a cytokine storm and hemophagocytic lymphohistiocytosis (HLH). In gene defects causing impaired T-cell activation/expansion, EBV-infected B cells persist and accumulate, leading to B-cell lymphoproliferative disorders (B-LPD/lymphoma). Furthermore, EBV can be reactivated increasing the risk of B-LPD.

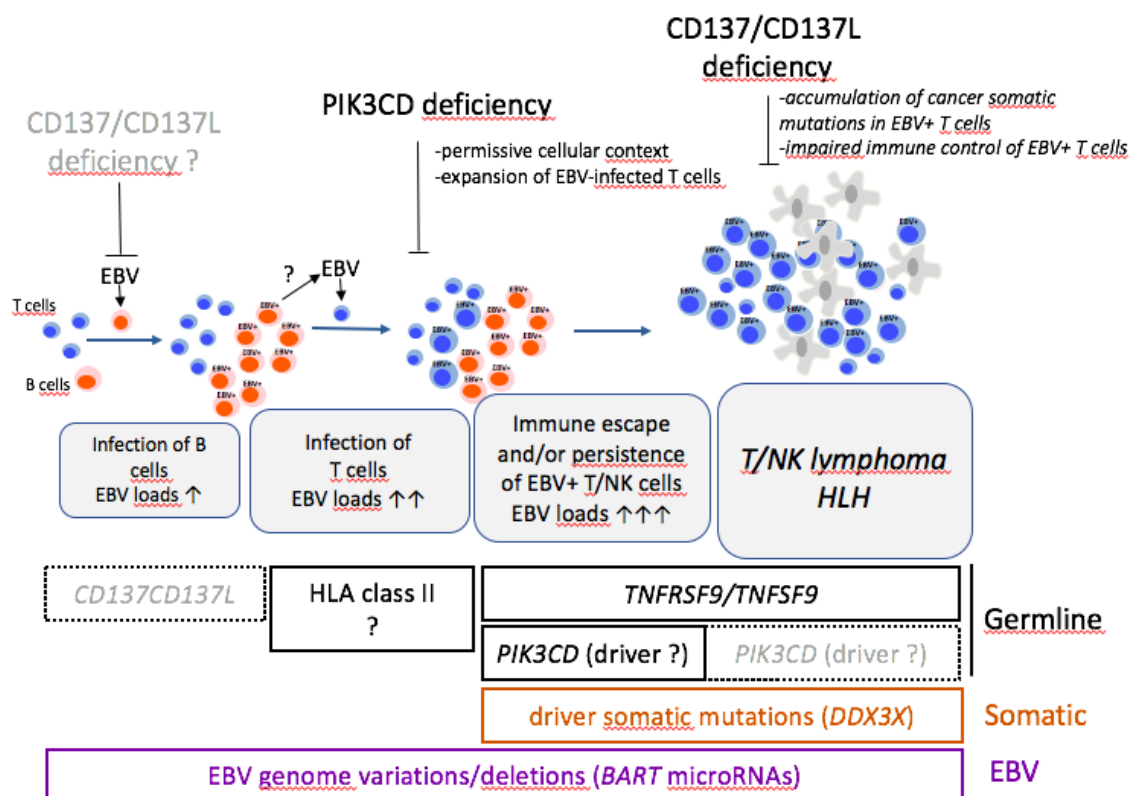


Figure 3. Hypothetical multi-step modelling of EBV-driven-cell lymphoproliferative T/NK CAEBV disorders based on Rodriguez et al. and Okuno et al. Sequential steps involved in the progressive development of T/NK LPDs from primary EBV infection of B cells to infection of T/NK cells, then evolving towards symptomatic CAEBV and further overt malignant T/NK lymphoma or HLH. Mature T/NK cells or NK/T lymphoid common progenitor are first infected and persist by acquiring driver somatic (DDX3X) and/or through germinal (PIK3CD) mutations that progressively may favor their expansion and increase their transformation. Specific genetic lesions of EBV genome (BART micro RNAs deletions) may also play an important role at this stage. EBV-infected T/NK cells escape at some point from the immune system due germinal mutations in components of immunity to EBV (*TNFRSF9/TNFSF9* coding CD137/CD137L also known as 4-1BB/4-1BBL) and may undergo to HLH or/and overt malignant transformation leading to lymphoma. Primary immunodeficiency could be also involved at the first step of the infection of B cells. In that setting, impaired immune control of infected B cells could increase EBV loads that may favor infection of T/NK cells. Selective infection of NK/T cells by EBV may also depend of germinal genetic factors including genes coding HLA-Class II.