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POINT-OF-VIEW

Destroy, what destroys you

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ABSTRACT

New evidence indicates the importance of CD137 for controlling Epstein-Barr virus (EBV) infections. (1) Mutations in *CD137* predispose to EBV-associated diseases. (2) EBV induces ectopic CD137 expression, thereby activating a negative feed-back regulation and reducing T cell costimulation. These findings suggest CD137 agonists as new treatments for EBV-associated diseases.

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CD137 (TNF-receptor superfamily 9 (TNFRSF9), 4-1BB) is a member of the tumor necrosis factor (TNF) receptor family, and a potent costimulatory molecule on activated T cells and NK cells. CD137 is also a promising target for tumor immunotherapy, as CD137 signaling greatly enhances immune responses against various cancers in human and mouse.[1,2] Agonistic antibodies against CD137 are being currently explored in several clinical trials for tumor immunotherapy.[3] Since only T cells that are activated via the T cell receptor express CD137, and since CD137 expression is transient, CD137 marks recently antigen-specifically activated T cells, and therefore CD137 agonists allow a comparatively specific costimulation of a T cell response.[4] CD137 is not only important for anti-cancer responses but also for generating effective immunity against viruses including, influenza, herpes simplex virus 1, lymphocytic choriomeningitis and vesicular stomatitis virus.[5]

CD137 ligand (CD137L) is expressed on antigen-presenting cells, and upon interaction with CD137L, CD137 triggers a T cell response via TNF-receptor associated factor 1 and 2 (TRAF1 and TRAF2), and activation of the transcription factor NF-κB.[6] CD137 costimulation increases proliferation, the secretion of IFN-γ and the cytolytic activity of T cells. These activities reflect CD137 being a main driver of cellular, type 1 helper T cell (Th1)-, type 1 cytotoxic T cell (Tc1)-polarized immune responses.[7]

Finally, after 30 years of research on CD137, three recent publications characterize 6 naturally occurring mutations in 8 patients in *CD137* which greatly enhance our understanding of the CD137 biology, and connect hitherto separated areas of research.

Alosaimi et al. identified a homozygous missense mutation in *CD137* in two unrelated patients with recurrent infections, persistent Epstein-Barr virus (EBV) viremia, and EBV-induced lymphoproliferation. The mutation abolished surface expression of CD137 on activated T cells, resulting in a diminished proliferation, IFN-γ secretion, perforin expression and a reduced cytotoxic activity of $CD8^+$ T cells upon stimulation with allogeneic and human leukocyte antigen (HLA)-matched EBV-transformed B cells.[8]

Somekh et al. analyzed four different patients with four distinct homozygous mutations in *CD137* that reduced or abolished CD137 expression. Defects were seen in T cell activation and in the diversity of the TCR repertoire. For one patient, the authors proved that the mutation in CD137 was the cause of the reduced T cell proliferation capacity by a gene rescue experiment, i.e. by transducing wild type CD137 into the patient T cells which restored activation-induced T cell proliferation.[9]

Rodriguez et al. identified two siblings with a homozygous mutation in *CD137* that prevented CD137 protein expression. Both siblings suffered from a persistent high EBV viremia, with EBV mainly being present in T cells. While the older sibling had an additional homozygous mutation in *PIK3CD*, a subunit of PI3K, and additional pathologies, no other mutations were found in the younger sibling, so that her pathology can be ascribed to the absence of CD137. T cells from the two siblings were unable to proliferate when cocultured with CD137L-expressing cells but upon lentiviral gene rescue of CD137 expression, CD137L-induced proliferation was restored. The authors conclude that the lack of CD137 costimulation prevented the clearance of EBV-infected T cells.[10]

Two of the four patients analyzed by Somekh et al. suffered from an EBV-related B cell lymphoma, Hodgkin's lymphoma (HL) and Burkitt's lymphoma, respectively, and one patient described by Alosaimi et al. presented with HL.[8,9] One patient analyzed by Rodriguez et al. suffered from EBV-associated T cell lymphoproliferative disease, and finally succumbed to hemophagocytic lymphohistiocytosis (HLH).[10] Thus, a common denominator of the three studies is that mutations in CD137 facilitate EBV-associated diseases, implying that under normal conditions CD137 limits EBV in causing pathology. This notion is supported by the diminished cytotoxic T cell response against EBV-infected B cells in the two patients analyzed by Alosaimi et al.[8]

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The 8 patients were inflicted by infections from a range of different pathogens which varied among the patients. But the common denominator is EBV. 7 of the 8 patients suffered from EBV viremia and the 8th patient suffered from Burkitt's lymphoma, an EBV-associated malignancy. This indicates that CD137 is essential for controlling EBV, and that the CD137-costimulated T cell response is a major reason why EBV is latent and asymptomatic in the vast majority of infected people. It can also explain the fact that EBV-associated diseases are comparatively rare, considering that 90% of the world population is infected.

CD137 is expressed by follicular dendritic cells [11] and follicular helper T cells (Tfh) [12] implying a function in B cell affinity maturation and development. Accordingly, all 4 patients analyzed by Somekh et al. had increased proportions of immature B cells and decreased proportions of memory B cells and plasmablasts and abnormal immunoglobulin levels.[9] This phenotype was also observed in the two patients analyzed by Rodriguez et al., where memory B cell numbers were severely reduced.[10] Although B cell subsets were not analyzed by Alosaimi et al., low IgG and high IgM levels, and a poor antibody response to tetanus toxoid and an absent recall response to pneumococcal polysaccharide vaccine, in one of the two patients indicate a disturbance in B cell maturation. Somekh et al also analyzed Tfh numbers and found reduced counts in 3 of the 4 patients.[9] These findings clearly document the importance of CD137 – CD137L interaction for the humoral immune response.

The TNF and TNF receptor families, to which CD137L and CD137 belong, contain 19 and 27 members, respectively, which can be regarded as evidence that costimulation is redundant. Redundancy is certainly the case for more general functions such as enhancement of T cell proliferation and cytokine secretion. But the phenotype of the 8 patients suggests that CD137, and possibly some other costimulatory molecules, also have non-redundant, specialized functions.

This mosaic of partial redundancy and unique functions is exemplified by CD27 and its ligand, CD70, also members of the TNFR and TNF families, respectively, which share many features with CD137 and CD137L such as T cell costimulation. But *CD27* and *CD70* are also essential for immunity against EBV, and homozygous mutations in them lead to EBV-associated diseases ranging from to HLH to HL.[13]

The emergence of EBV viremia and EBV-associated disease in homozygous CD137-deficient patients stands seemingly in contrast to earlier studies demonstrating that EBV, via its Late Membrane Protein 1 (LMP1), induces CD137 expression in NK/T-cell lymphoma (NKTCL),[14] and in HL,[15] two malignancies that are associated with EBV. HL is driven by the malignant Hodgkin and Reed-Sternberg (HRS) cells, which in most cases are derived from B cells. Even though CD137 is rarely found on healthy B cells, CD137-expressing HRS cells could be identified in 86% of HL cases.[16,17] These 86% of CD137-expressing HRS cells are significantly higher than the estimated 30 – 50% of HL cases that are associated with EBV, indicating there may be additional factors that induce CD137 expression in HRS cells. Nevertheless, the 86% of CD137-expressing HRS cells is too high a number to be due to coincidence, and indicates that EBV gains a growth and/or selection advantage by inducing expression of CD137.

And indeed, the ectopic expression of CD137 on infected cells enables EBV to hijack a physiological negative feed-back regulation for CD137 that allows it to inhibit T cell costimulation by CD137.[16,18,19] Therefore, both scenarios, (1) mutation of CD137 and (2) ectopic expression of CD137 achieve the same end, the downregulation of T cell costimulation through CD137, i.e. to disable an immune pathway that limits EBV propagation. In addition, engagement of CD137 on HRS cells induces them to secrete IL-13, a major growth factor for HRS cells and HL.[20]

Induction of ectopic CD137 expression is just one of several mechanisms that EBV uses to escape immune surveillance. Alternative mechanisms include interfering with the MHC class I and class II antigen presentation pathways to avoid recognition and subsequent elimination by $CD4^+$ and $CD8^+$ T cells. The EBV-encoded lytic protein BNLF2a inhibits the transporter associated with antigen processing (TAP)-mediated peptide transport by preventing cytosolic viral peptides and ATP from binding to TAP complex.[21] Other lytic proteins that reduce surface expression of MHC class I include BGLF5 and BILF1.[21] The interference of viral peptide loading onto MHC class I molecules and curtailed surface expression of MHC class I decrease peptide-MHC presentation to $CD8^+$ T cells, thereby averting cytotoxic $CD8^+$ T cell mediated lysis. The latent EBV protein EBNA1 adopts a different strategy to evade immune detection. An internal glycine-alanine repeat domain (GAr) within EBNA1 decreases the rate of translation of EBNA1 mRNA and prevents proteosomal degradation to peptides.[21] The ability to regulate EBNA mRNA synthesis and production of viral peptides helps EBV-infected cells to avoid recognition.

There are several other immune escape mechanisms employed by EBV, among them EBV-encoded microRNAs (miRNA), which have been shown to control gene expression of MHC class II and lysosomal enzymes IFI30, LGMN and CTSB involved in MHC class II peptide processing.[22] EBV miRNAs also repress the secretion of the pro-inflammatory cytokine IL-12 as a means to suppress $CD4^+$ Th1 differentiation.[22] Further, the EBV immunoevasin vIL-10, a viral homolog of human IL-10, decreases expression of the costimulatory molecules CD86 and CD80 in EBV-infected monocytes as well as the production of pro-inflammatory cytokines. EBV also targets secretion of interferons to circumvent anti-viral responses.[21]

An additional or alternative way by which EBV could have inhibited CD137 costimulation would have been the secretion of soluble CD137 [23] which competes with cell surface-expressed CD137 for CD137 ligand, and reduces T cell costimulation.[24,25] But while levels of sCD137 are high in chronic lymphocytic leukemia they are not elevated in HL.[26]

CD137 is strongly expressed on regulatory T cells (Treg),[27] and a high frequency of $CD137^+$ Treg correlated with poor prognosis in lung adenocarcinoma patients.[28] This is in line with $CD137^+$ Treg being more suppressive than $CD137^-$ Treg.[27] Can the lack of CD137 on Treg be responsible for the observed phenotype of the patients? Although Somekh et al. report lower Treg frequencies in the patients[9] it is unlikely that the observed EBV-associated lymphoma is due to fewer or less functional Treg.

Further, CD137-CD137L interaction induces a bidirectional signaling. Reverse signaling by CD137L has been described as

a signal transduction of CD137L into the cells it is expressed on.[29,30] This reverse CD137L signaling would also be affected in the absence of CD137. Since reverse CD137L signaling enhances APC activity, it contributes to the immune stimulation by CD137 forward signaling which costimulates T cell activity and activates NK cells.[1,2] It is therefore possible that the observed phenotypes, or aspects of them, are due to a combined lack of CD137L reverse signaling and CD137 forward signaling.

The bright side of these new insights is that EBV has given away what is dangerous to it, which seems to be a CD137-costimulated immune response. On the basis of this knowledge, CD137 agonists should be explored for treatment of EBV-associated malignancies such, as NKTCL, Burkitt's lymphoma and HL.

Abbreviations

EBV	Epstein-Barr virus
HL	Hodgkin lymphoma
HLH	Hemophagocytic lymphohistiocytosis
HRS cells	Hodgkin and Reed-Sternberg cells

Disclosure of Potential Conflicts of Interest

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