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EBV the prototypical human tumor virus—just how bad is it?

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EBV was the first candidate human tumor virus. It is found in several human cancers, particularly lymphomas and carcinomas, and has potent transforming activity *in vitro*. Yet the virus persists benignly for the lifetime of more than 90% of the human population. Thus it seems that EBV has the potential to be highly pathogenic yet rarely manifests this potential. Studies over the last several years show this is because the virus actually persists in resting memory B cells and not proliferating cells. EBV needs its growth-promoting ability to gain access to the memory compartment but has evolved to minimize its oncogenic potential. These studies also reveal that the different EBV-associated tumors apparently arise from different and discrete stages in the life cycle of B cells latently infected with EBV. This raises the question of how actively EBV participates in the development of human tumors. Does the virus cause the disease, or is it simply a passenger? In the case of immunoblastic lymphoma in the immunosuppressed patient, the virus almost certainly plays a causative role, but in other cases, such as Burkitt's lymphoma, the contribution of EBV remains less clear. (J Allergy Clin Immunol 2005;116:251-61.)

Key words: *Epstein-Barr virus, carcinoma, lymphoma, persistent infection, latency, B cell, memory*

EBV is well known because of its characteristic biology.[1-3] If you define the success of a pathogen by the number and extent of hosts it infects, EBV is the most successful human pathogen because it latently infects virtually the whole human population and persists for life.[4] In tissue culture EBV is one of the most potent transforming viruses,[5,6] and it is found in several human cancers,[1,3] yet for most of the population, it remains benign. The collection of viral latent proteins expressed is different in each tumor type (Table I). Sometimes all of the known latent proteins are expressed, sometimes a limited subset, and sometimes only one.

Abbreviations used

BL: Burkitt's lymphoma
CTL: Cytotoxic T lymphocyte
EBNA: EBV nuclear antigen
HD: Hodgkin's disease
IM: Infectious mononucleosis
LMP: Latent membrane protein
NPC: Nasopharyngeal carcinoma
PTLD: Posttransplantation lymphoproliferative disease

Despite the apparent robustness with which the human population deals with EBV (>95% of all adults carry the virus), the diseases caused by EBV indicate that the situation is finely balanced. The first indication comes from X-linked lymphoproliferative disease.[7] In this disease persistent infection is not established because mutations in the SH2D1A gene[8,9] cause acute EBV infection to become a fatal disease. Put melodramatically, a single nucleotide change in the SH2D1A gene is all that prevents the vast majority of the human race from dying of acute EBV infection.

The second indication comes from the observation that immunologic disturbance, as a predisposing factor, is a unifying theme for all of the EBV B-cell lymphomas. This also suggests that the regulation of EBV infection in B cells is finely balanced. Disruptions can lead to deregulation and EBV-driven tumor development, even in otherwise healthy carriers of the virus. The clearest example of this is individuals who are immunosuppressed, such as patients undergoing organ transplantation, who are iatrogenically immunosuppressed, or patients with AIDS, who are immunosuppressed by HIV. These individuals are at risk for EBV lymphomas that are aggressive and often fatal.[10] This means that it is only courtesy of an active immune response that we are protected from fatal EBV-driven lymphoma. Yet there are some curious properties of these tumors that suggest the risk is not as high as might be expected. For example, not every immunosuppressed

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TABLE I. The EBV transcription programs in normal B cells and tumors

Transcription program	Genes expressed*	Infected normal B-cell type†	Function	Infected tumor type
Growth	EBNA1, 2, 3a, 3b, 3c, LP, LMP1, LMP2a, and LMP2b	Naive	Activate B cell	Immunoblastic lymphoma
Default	EBNA1, LMP1, and LMP2a	Germinal center	Differentiate activated B cell into memory	HD
Latency	None	Peripheral memory	Allow lifetime persistence	
EBNA1 only	EBNA1	Dividing peripheral memory	Allow virus in latency program cell to divide	Burkitt's lymphoma
Lytic	All lytic genes	Plasma cell	Replicate the virus in plasma cell	

*Does not include the noncoding EBER and BART RNAs that are assumed to be ubiquitous but have not been rigorously identified in all of the infected subtypes.
†Except where indicated, the cell types are primarily restricted to the lymphoid tissue of the Waldeyer ring.

patient has the tumors, and the tumors are frequently oligoclonal. This is not the expected outcome. If it were simply a case of the immune system failing to control the EBV-infected cells, every immune-suppressed person should fill up with multiple tumors because everybody carries approximately 5×10^5 infected cells,[11] and immunosuppressed individuals carry perhaps 50 times more.[12]

Taken together, these observations raise several questions. How does EBV persist benignly for the lifetime of a human despite its pathogenic potential? Why does EBV have such potent and pathogenic properties if it has evolved to persist for the lifetime of the human host it puts at risk by manifesting those properties? Where do the EBV-associated tumors come from, why do they have different patterns of latent gene expression, and why does disruption of the immune system predispose to EBV lymphoma development? Lastly, what goes wrong in the maintenance of persistence that leads to EBV-associated diseases?

The key to answering these questions comes from a model of EBV persistence[13,14] developed from the observation that despite EBV's transforming ability, it persists *in vivo* in resting[15] memory[16] B cells that do not express any viral proteins.[17] This article will first briefly review the complete life cycle of EBV infection and then discuss how the origins of EBV-associated tumors can be explained in the context of this model, with special emphasis on the role of an impaired immune response. Finally, the model will be used to attempt to answer the questions posed above.

EBV PERSISTENCE *IN VIVO*

The essence of EBV's behavior is that under normal conditions, it does not aberrantly deregulate the behavior of infected B cells *in vivo*. It initiates, establishes, and maintains persistent infection by subtly using virtually every aspect of normal B-cell biology. Ultimately, this allows the virus to persist within memory B cells for the lifetime of the host in a fashion that is nonpathogenic. The thesis of this review is that EBV is not a natural tumor virus and that it has developed strategies to minimize its pathogenic potential to the host.

Establishment

To understand EBV biology, it is first necessary to understand the biology of the B lymphocyte in the mucosal lymphoepithelium of the tonsil (Fig 1). A summary of normal mature B-cell biology and the proposed parallels with EBV is given in Figs 2 and 3, and a summary of information on the different viral latency programs is presented in Table I. The model has been described in detail elsewhere.[13,14]

The normal B-cell response. Environmental antigens entering the mouth are continuously sampled by the epithelium of the tonsil. Underneath the epithelium is a bed of lymphoid tissue including large numbers of naive lymphocytes.[18,19] If antigen is recognized by the antibody on the surface of the naive B cell, it will bind and cause the B cell to become an activated blast and migrate into the follicle to form a germinal center (Fig 2).[20] Here the cell undergoes rounds of rapid proliferation associated with isotype switching and mutation of the immunoglobulin genes, followed by competitive selection for those with the antibody that binds the antigen best. Those who lose in the competition to bind antigen die by apoptosis. Ultimately, the surviving cells leave the germinal center as memory cells primed to make a rapid response to rechallenge with the antigen. This process requires, in addition to the antigen, a signal to the B cell from an antigen-specific T helper cell.

The parallel with EBV. EBV also transits the epithelium and infects naive B cells[21] in the underlying tissue, where it expresses a set of latent genes that cause the cell to become activated and proliferate as though it were responding to antigen. This EBV transcription program (the growth program, Fig 2) involves 9 latent proteins, including nuclear antigens (EBV nuclear antigens [EBNAs]) and membrane proteins (latent membrane proteins [LMPs]).[2] These proteins have all the necessary activities to push the B cell to become an activated blast without any necessity for external signaling. This cell migrates to the follicle, where the viral transcription program changes,[22] such that

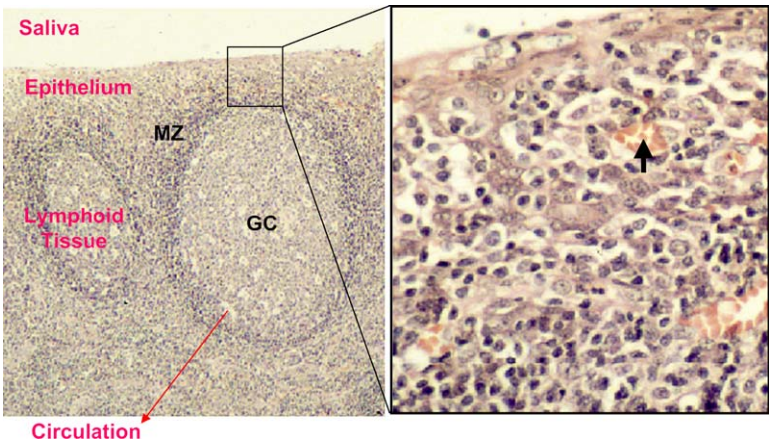

FIG 1. The lymphoepithelium of the palatine tonsils from the Waldeyer ring. The tonsil consists of a highly involuted epithelium, creating a large surface area with deep invaginations. The epithelial surface is at the top of both micrographs. Antigen and EBV both enter through saliva and cross the epithelial barrier to activate or infect, respectively, the naive B cells below. The mantle zone *(MZ)*, containing naive B cells (dark blue), is always facing the surface and is continuous with the epithelium. Naive cells enter the tonsil *(black arrow)* through the high endothelial venules (orange cuboidal cells). Numerous follicles containing germinal center B cells *(GC)* are arranged parallel to the surface. B cells leave the germinal center *(red arrow)* and enter the circulation through the efferent lymphatics. A higher magnification *(expanded box)* reveals the sponge-like structure of the epithelial cells in the lymphoepithelium that create spaces extending all the way to the mantle zone that are filled with infiltrating lymphocytes such that there is frequently only a single epithelial cell between the outer surface and the lymphocytes. (The micrographs were kindly provided by Dr Marta Perry).

only 3 of the latent proteins are expressed: EBNA1 (required to replicate the viral DNA) and 2 membrane proteins, LMP1 and LMP2 (the default program, Fig 2).

The functions of LMP1 and LMP2 have evolved to steer the latently infected B cell through the germinal center environment. LMP2 alone will push B cells to form a germinal center in the mucosal follicle[23]; LMP1 and LMP2 can drive immunoglobulin gene mutation[23] and isotype switching[24] (the defining markers of the germinal center), respectively, and LMP1 downregulates expression of the germinal center regulatory transcription factor bcl-6,[25] the signal for a memory cell to exit the germinal center.[26] This implies coordinated expression of LMP1 and LMP2, where LMP2 is turned on before and LMP1 is turned on during the germinal center reaction. Thus constitutive expression of LMP1 in the absence of LMP2 blocks germinal center formation because the cells can never turn on bcl-6, an essential step in germinal center formation.[27]

This explains why EBV has the ability to make cells proliferate, despite the fact that this puts the host at risk for neoplastic disease. Essentially it has to because this is the mechanism, activation followed by differentiation, by which a normal B cell enters the B-cell memory pool.

Maintenance

Once in the periphery, the latently infected cells shut down all viral protein expression (the latency program) and appear to be maintained as normal memory B cells.[17] In the early stages of acute infectious mononucleosis (IM; primary EBV infection in the adult), the number of such cells in the blood can reach staggering proportions, with 50% or more of all memory cells being infected.[28] However, the numbers decrease rapidly (half-life of 7 days; Hadinoto and Thorley-Lawson, unpublished data) for the first 2 months and then more steadily after that, until by 1 year there are typically only about 1 in 10^5 to 10^6 infected memory B cells. After this time, the level of infected cells appears to be relatively stable over many years.[11] This presumably represents a balance between the replenishment of latently infected memory cells through cell division[17] and their loss through viral replication (see below). This cell division must be regulated as part of normal memory B-cell homeostasis because there are no viral proteins expressed that could cause the cell to divide. When they divide, they express EBNA1 (the EBNA1-only program, Fig 2),[17] which is needed to allow the viral DNA to replicate with the cells.[29] Perhaps not surprisingly, because EBNA1 represents the only point of immune attack of the memory cells, EBNA1 has evolved to be poorly recognized by the immune system.[30]

By gaining entrance to normal memory B cells and shutting down viral protein expression, the virus is safe from immune surveillance. It is also benign because none of the latent proteins that drive growth are expressed. This explains why EBV is able to persist benignly in the vast majority of human subjects: EBV infection *in vivo* does not drive limitless proliferation. Rather it drives transient proliferation so that the cells can become resting memory cells. The virus persists in nonpathogenic resting cells not proliferating blasts. This also explains why EBV-associated tumors do not arise in every infected individual, even when they are immunosuppressed; something must go wrong with the normal biology that takes the latently

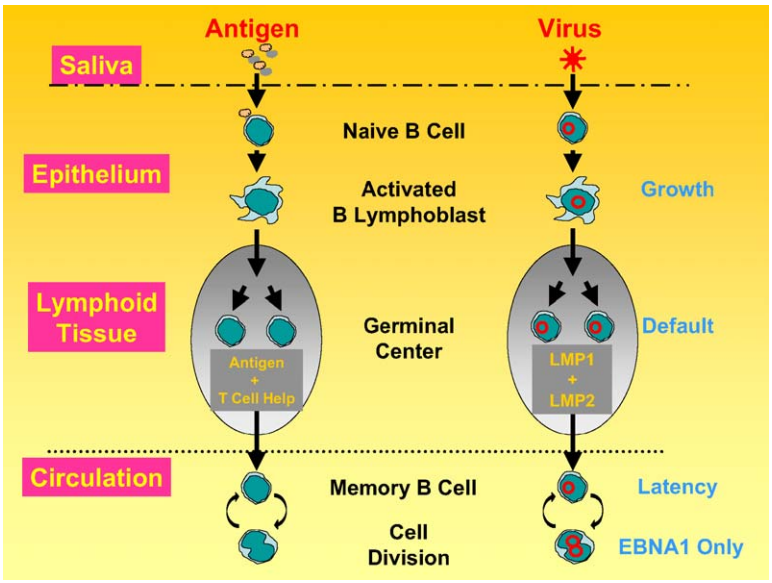

FIG 2. A model of how EBV uses normal B-cell biology to establish and maintain persistent infection in memory B cells. The response of a normal B cell to antigen, leading to the production of antigen-specific memory cells in the peripheral circulation, is diagrammed to the left, and the parallel series of steps by which EBV establishes latent infection in peripheral memory B cells is shown to the right. The specific viral transcription programs are labeled in blue to the right. For details, see the text and Table I.

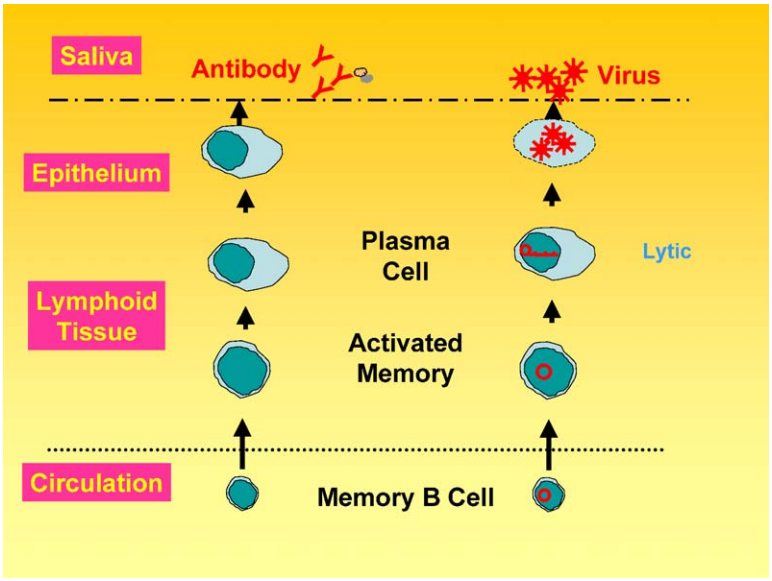

FIG 3. A model of how EBV uses normal B-cell biology to replicate and be shed into saliva. The pathway by which antigen-specific B cells become activated and differentiate into antibody-producing plasma cells is shown to the left, and the parallels that lead to shedding of EBV are shown to the right. The EBV transcription program is indicated in blue to the right. For details, see text and Table I.

infected cells into a resting state before EBV could be involved in tumor development.

Release

By accessing the memory compartment, EBV has a site for long-term persistence. However, it must replicate and be shed to spread to new hosts. The parallels between normal B-cell biology and the mechanism of viral shedding are shown in Fig 3. Signals that cause the B cell to differentiate into an antibody-secreting plasma cell will in turn reactivate the virus.[31] Because antibody-secreting plasma cells migrate into the mucosal epithelium,[18,32] such a cell will be perfectly placed to release virus onto the mucosal surface, which, in the case of the tonsils,

is saliva. Thus infectious virus is spread through saliva contact.[33]

Epithelial cells and viral shedding

Although EBV is considered to be a B-lymphotropic virus, it can also infect epithelial cells because it is found in several important diseases of epithelial cells, including nasopharyngeal[34] and gastric[35] carcinomas and oral hairy leukoplakia.[36] What is less clear is whether epithelial cells play a role in the normal biology of EBV. Early reports that claimed to find EBV in healthy nasopharyngeal epithelium have been discredited[37]; however, recent work has revisited this possibility. There is now evidence that normal epithelial cells in the nasopharynx express a distinct EBV receptor,[38] that they can be infected *in vitro*, and that they are infected *in vivo*.[39] However, it remains undetermined whether this infection occurs fortuitously because this epithelium is an area in which EBV happens to replicate or because it is an important component of the viral biology. The most likely role for epithelial cells is as a site for replication and amplification of the virus rather than as a site of persistent latent infection.[40,41] Because the receptor is only expressed on the basolateral surface of epithelial cells, the virus can only infect from the lymphoid tissue and not from saliva. Thus if epithelial cells play an amplification role, it is during viral shedding and not primary infection. Perhaps the most compelling indirect evidence for epithelial cell infection comes from simple numbers. Estimates of the number of lymphocytes replicating EBV in the tonsils[42] indicates that there are not nearly enough to account for the rates of viral shedding found in saliva (Hadinoto and Thorley-Lawson, unpublished data). This suggests that there must be a location-mechanism for amplifying the virus shed from plasma cells. The obvious candidates are epithelial cells because, from studies on oral leukoplakia, we know that epithelial cells replicate EBV to high copy numbers.

Unresolved questions about persistence in memory cells

There are important unresolved questions relating to EBV persistence in memory B cells.

First, what is the relative contribution of reinfection versus homeostatic cell division to the maintenance of stable levels of latently infected cells? We know that the host mounts a massive cytotoxic T-cell response against cells replicating EBV and newly infected cells[43] and a neutralizing antibody response against the virus.[4] It is therefore unclear whether newly infected cells are produced rapidly enough and survive long enough to contribute to the pool of latently infected memory cells once the immune response has begun. It is conceivable that new infection is only critical in establishing the pool of latently infected memory cells before the onset of the immune response and thereafter plays no role. A clue that this might be true comes from the observation that the epitopes recognized by cytotoxic T cells on newly infected B cells are conserved.[44] Usually, a virus is continuously varying its sequence to avoid the immune response (eg, HIV[45]), but in the case of EBV, it seems to ensure that newly infected cells are rapidly destroyed. This suggests that the new infection route might only be viable before the immune response arises (ie, in acute infection). Thereafter, the virus depends on homeostasis of the pool of latently infected memory cells for persistence and ensures that any new infected cells are rapidly killed because they might pose a lymphoproliferative threat to the host.

Second, if EBV persists in normal antigen-selected B cells (unpublished results), why does it have LMP1 and LMP2, which can replace all the signaling necessary to produce a memory B cell? The answer to this is not yet clear. One possibility is that the role of LMP1 and LMP2 might be to give a selective advantage to the virus-infected cells in the highly competitive environment of the germinal center. This would give the latently infected cell a better chance of making it into the memory pool.

Third, why does EBV not infect memory cells directly? *A priori* there seems no reason why EBV could not use the same mechanism to drive an infected memory cell back into memory; however, the evidence does not favor this alternative. First, there is no evidence that direct infection of memory cells occurs consistently *in vivo*.[21,22] Second, when it does occur, it seems to lead to clonal proliferation[46,47] and not differentiation, and third, the pool of latently infected memory cells is skewed (Sousa and Thorley-Lawson, unpublished data), which would not be expected if EBV infected memory cells at random. One possible explanation comes from the known biology of B cells. Activation of naive B cells through the germinal center leads predominantly to the production of memory cells over plasma cells,[48] whereas activation of memory cells leads predominantly to the production of plasma cells.[49] Therefore if the goal of EBV is to access the memory compartment, it will do so more efficiently by infecting and activating naive B cells rather than memory cells.

EBV AND DISEASE

General considerations

EBV has been associated with a number of human diseases. These generally fall into 2 categories: autoimmunity and cancer.[1,3] The idea that EBV might be involved in autoimmunity stems from the knowledge that the virus can infect any B cell and cause it to proliferate indefinitely in culture. This raises the possibility that EBV could immortalize forbidden clones of B cells *in vivo*, perhaps allowing them to produce autoimmune antibodies in an uncontrolled fashion. This philosophic underpinning for a role of EBV in autoimmunity can now be seen to be incorrect. We know that EBV does not persist *in vivo* by immortalizing B cells but by establishing a true latency in normal resting memory B cells. There are also technical difficulties to proving a causal role for EBV in these diseases. First, EBV persists in circulating memory cells and therefore will be found in all tissues, irrespective of disease causality. Second, it is now apparent

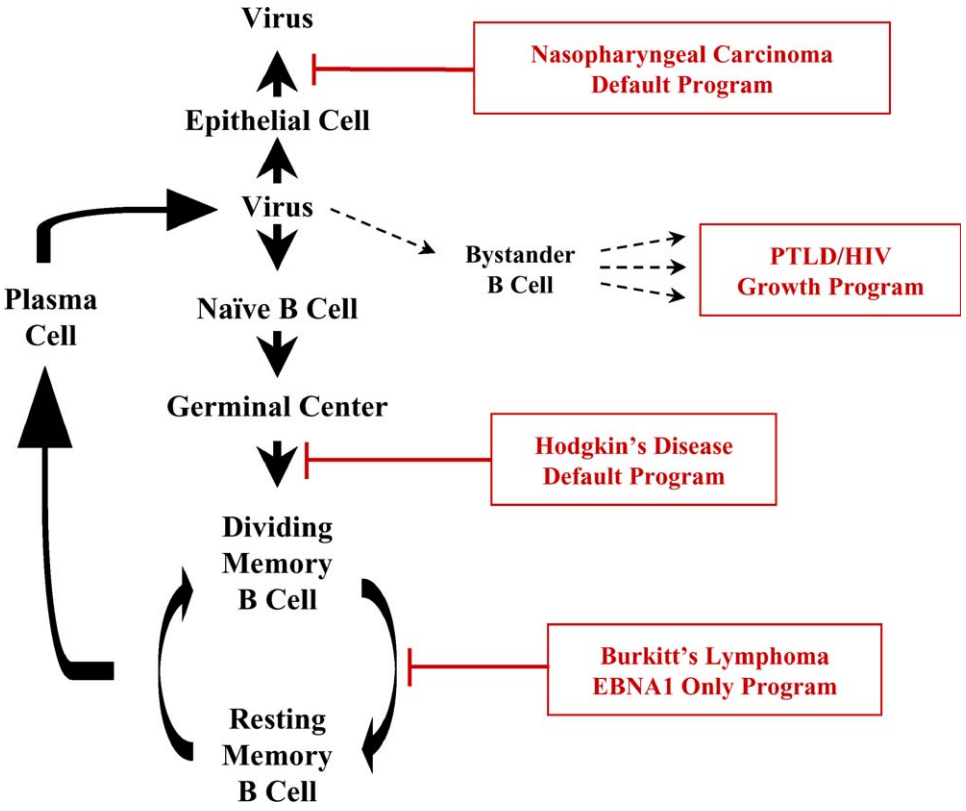

FIG 4. The putative check points in the EBV life cycle that give rise to tumors. The events that occur normally in healthy carriers are denoted in black. For details, see Figs 2 and 3. EBV normally infects naive B cells in the Waldeyer's ring, and these cells can differentiate into memory cells and out of the cell cycle *(thick arrows)*, and therefore they are not pathogenic. *PTLD*: If a cell other than the naive B cell in the Waldeyer ring becomes infected, it will express the growth program and continue to proliferate because it cannot differentiate out of the cell cycle *(thin dashed arrows)*. This is a very rare event, highlighting how carefully controlled EBV infection is. Normally, these bystander B-cell blasts would be destroyed by CTLs, but if the CTL response is suppressed, then they can grow into PTLD. Note: a bystander-type cell could also arise if a latently infected germinal center or memory cell fortuitously switched on the growth program. *Hodgkin's disease* arises from an EBV-infected cell that is blocked at the germinal-center cell stage. This results in constitutive expression of the default program. *Burkitt's lymphoma* evolves from a germinal-center cell that is entering the memory compartment but is stuck proliferating. Consequently, the cell expresses EBNA1 only. *Nasopharyngeal carcinoma* is hypothesized to arise from a latently infected epithelial cell blocked from terminal differentiation and viral replication. It is unclear why these cells would express the default program.

that EBV is extremely sensitive to the state of the immune system. This is because it relies on normal B-cell biology to establish and maintain persistence and T-cell responses to modulate the level of infection. Changes that affect the functionality of the immune system affect EBV by changing overall viral loads and states of infection. Because autoimmune diseases classically disrupt the immune system, it will be extremely difficult to dissect out causality of EBV from the background noise of changes occurring in the virus because of the disease. For all of these reasons, it has been difficult to establish a clear connection between EBV and any autoimmune diseases. Currently, such associations remain speculative, controversial, or both.

The reason to believe EBV might cause cancer is apparent. EBV encodes genes that make B cells grow. Such genes will, of their nature, have potential as oncogenic risk factors. However, as described above, EBV has evolved to minimize the risk that an infected cell will proliferate out of control. Therefore something must go wrong with the normal viral biology for EBV to play a causative role in tumor development. The plausibility of EBV as an oncogenic virus has led to claims of its association with many human tumors. Some, such as breast and hepatocellular carcinoma, have never been substantiated, but there are now several for which strong evidence exists, including immunoblastic lymphoma in immunosuppressed patients, Burkitt's lymphoma, Hodgkin's disease (HD), and nasopharyngeal carcinoma (NPC). The origins of all of these tumors can be understood as arising from specific stages in the EBV life cycle (Fig 4) and appear to be associated with disturbances of the immune system. This begs the following question: How convincing is the evidence that EBV

plays a causative role in these tumors and is not simply a passenger in a tumor cell that arose from an infected cell type?

Lymphoma in the immunosuppressed

Individuals who are immunosuppressed are at risk for development of B-cell lymphoproliferative diseases, such as the immunoblastic lymphomas in patients with AIDS and the posttransplantation lymphoproliferative diseases (PTLDs) in patients undergoing organ transplantation.[10] These are a heterogeneous collection of disorders that usually carry the virus and express the growth program (Table I).[50] A wide range of factors (eg, organ type, immunosuppressive regime, location, and donor origin) influence the frequency with which these tumors arise. The explanation usually given for the origin of these tumors is that immunosuppression of the cytotoxic T-lymphocyte (CTL) response to EBV allows uninhibited growth of EBV-infected cells; however, it is not that simple.

From the discussion above on the mechanism of EBV persistence, it is apparent that, under normal conditions, infected naive B cells in the tonsils do not give rise to lymphoma because they differentiate out of the cell cycle to become resting memory cells. For a cell to express the growth program, survive, and evolve into a neoplasm, 2 events must occur: the EBV-infected cell must be unable to respond to signals that drive it to differentiate into a resting memory cell, and the CTL response must be crippled so that these lymphoblasts can continue to proliferate. This could occur if any B cell that is not a naive B cell in the tonsil is exposed to the virus by chance—bystander infection (Fig 4). It could also occur if a latently infected germinal center or memory cell fortuitously received signals that caused it to inappropriately turn on the growth program. These cells can not exit the growth program, and therefore they continue to proliferate. Normally, they would be rapidly eliminated by CTLs because of the conserved CTL epitopes they express (see above); however, in the absence of effective T-cell immunity (immunosuppression), they will continue to proliferate. Direct evidence that this is indeed the case comes from studies of tonsils from acutely infected individuals. In these tonsils clonal expansions of directly infected germinal center[51] and memory cells[46] driven by the growth program can be found. Because these are bystander-infected cells, they are unable to differentiate into resting memory cells. Consequently, they proliferate until the immune response arises to eliminate them, explaining why such clones are never seen in healthy carriers of the virus but will appear if the immune response is subsequently suppressed.

The origin of these tumors also explains their heterogeneity. They are derived from a mixture of B-cell types[52] consistent with arising from a variety of bystander B cells that get infected by chance and not a specific subset of infected cells. This also explains why the tumors are relatively rare. The vast majority of infected cells differentiate into a resting memory state because they are naive; they will not be a cancer risk. Only the rare, atypical bystander infection is a risk for tumor development.

Hodgkin's disease

Acute EBV infection in the adolescent-adult can give rise to IM, long known to be a risk factor for HD. However, the strongest evidence directly linking EBV with HD came with the finding that approximately 40% of the tumors contain clonal EBV,[53] which can approach 80% in developing countries and up to 100% in AIDS-related HD.[54] In addition, the tumor cells express the default transcription program (Table I),[55-58] which includes 2 proteins (LMP1 and LMP2) that deliver survival and growth signals,[59-61] at least one of which (LMP1) is known to act as an oncogene.[62] A characteristic of IM, compared with the subclinical infection seen in children, is profound disruption of the immune system.[63] This includes massive levels of virus-infected memory B cells ($\geq$50%), a striking T-cell lymphocytosis caused, at least in part, by a very aggressive cytotoxic T-cell response, and tissue damage in the lymph nodes. The disease is almost certainly a product of an overreactive inflammatory response, and B-cell function is so badly disrupted that one of the characteristics of IM is the production of a broad range of nonspecific, low-affinity, so-called heteropohile antibodies. This suggests that HD is the consequence of deregulated EBV infection caused by the severe immunologic disturbance of IM. Nevertheless, the possibility that EBV is a passenger cannot be excluded. If the immunologic disruption of IM alone is the risk factor for HD, it is possible that the premalignant B cell will have EBV in it simply by chance.

There is good evidence that EBV-positive HD arises from an infected germinal-center cell. As discussed above, one of the characteristics of germinal-center cells is that they actively mutate their immunoglobulin genes in a process termed hypermutation, which leaves a characteristic pattern of mutations. The immunoglobulin genes of HRS cells have this pattern of mutation.[64] In addition, the default transcription program is used by EBV in latently infected germinal center B cells.[22] Thus the immunoglobulin mutations and the viral gene expression data independently support the idea that EBV-positive HD arises from an EBV-infected germinal center B cell (Fig 4).

Burkitt's lymphoma

EBV was discovered in cultured tumor cells from patients with the endemic form of Burkitt's lymphoma (BL).[65] It is sobering to realize that 40 years later, we still do not know how or even for sure whether EBV causes BL. This is despite the large volume of information we have acquired about EBV's molecular and cellular biology, immunology, virology, epidemiology, clinical manifestations, and disease associations.[1-3] The most compelling evidence of EBV's involvement in BL is the high frequency (98%) of tumors carrying the virus[66] in endemic areas and the presence of clonal EBV in all of the tumor cells.[67] However, none of the growth-promoting latent genes are expressed. The only genes expressed

encode for EBNA1[68] and the untranslated RNAs called EBERS and BARTS. It has been suggested that EBNA1[69] and the EBERS[70] might have oncogenic potential, but the findings remain unsubstantiated and are controversial. Consequently, there is currently no broadly accepted understanding of the role of EBV in BL.[71-74] What is apparent, however, is that malaria, which is chronically immunosuppressive,[75] is classically known to be a risk factor for endemic (ie, EBV-positive) BL development.[76] Once more, this supports the notion, discussed throughout this review, that EBV infection in the context of a compromised immune system is the risk factor for lymphoma development.

Using the same arguments as for HD, we can surmise that BL is a tumor cell of a proliferating, latently infected memory B cell (Fig 4). BL has the same pattern of immunoglobulin gene hypermutations as memory B cells,[77] and there is only one way known for producing an EBNA1-only phenotype in nontumor cells. This is when a latently infected memory cell expressing the latency program divides as part of normal B-cell homeostasis (Fig 2).[17] One property of BL inconsistent with this idea is that the tumor cells have the surface phenotype of germinal-center cells.[78] However, the cellular phenotype of tumor cells can be misleading. This is exemplified by HD, which is generally thought to be derived from a germinal-center cell, although it bears no phenotypic or morphologic resemblance to such cells. Thus it is difficult to know how directly the final cellular phenotype of BL relates to the original infected precursor. Possibly, BL is derived from a germinal-center cell on its way to becoming a resting memory cell expressing the latency program but through tumor-driven growth continues to proliferate and therefore expresses the EBNA1-only phenotype.

Nasopharyngeal carcinoma

Given the B lymphotropism of EBV, it is surprising that one of the best candidates for a tumor caused by EBV is not a lymphoma but a carcinoma, NPC, responsible for 20% of all cancers in China and Taiwan[79] and therefore an important world health problem. Virtually 100% of undifferentiated NPCs worldwide contain clonal EBV.[34,80] The tumors express the viral default transcription program.[81-83] Although only a subset, approximately 40%, express LMP1, it has been reported that the premalignant lesions of NPC all express LMP1.[84] As with HD, the presence of LMP1 and LMP2 is additional evidence that the virus is playing a part in the cause of the tumor. Because LMP1 and LMP2 are potently and specifically evolved B cell–signaling molecules, their presence in the epithelial cells of NPC suggests the virus might be there fortuitously. An example of this is LMP2, which functions to cause B cells to migrate into mucosal follicles.[23] This migratory ability, expressed in epithelial cells, might result in the invasive and metastatic activity of NPC.

The potential role of EBV in NPC is clouded by our lack of certain knowledge about the role of epithelial cells in EBV biology. In Fig 4 the speculative assumption is made that EBV latently infects epithelial cells that then proceed to replicate the virus and shed it into saliva. NPC would derive from such a latently infected, undifferentiated epithelial cell, which was blocked from switching to viral replication and therefore continues to be latently infected. Why the default program, usually found in germinal center B cells, is expressed in NPC is completely unclear.

CONCLUSION: ANSWERS TO THE QUESTIONS

In the introduction to this article, several questions were raised about EBV. The answers to these questions can now be explained in light of the discussion above.

First, why does EBV make cells proliferate when it puts the host at risk for neoplastic disease?

Because it has to. The newly latently infected naive B cell has to become an activated blast before it can differentiate into a resting memory cell.

Second, where do the EBV-positive tumors come from, why do the different tumors express different viral latent gene transcription programs, and why is disruption of the immune system a risk factor? The virus uses these different transcription programs to manipulate the biology of the infected B cell so that it can gain entry into and then persist in memory B cells. Any disruption of the immune system that interferes with the ability of the EBV-infected cells to become a resting memory cell will increase the risk of tumor development. Each tumor derives from a different step in this process and represents a cell that is blocked from progressing into a resting state and therefore continues to express the viral transcription program of its progenitor.

Third, why are there so few EBV-infected tumors in the human population, even with immunosuppression, despite the large numbers of EBV-infected cells in each individual and the ability of EBV to make lymphocytes grow? This is because the viral biology is tightly regulated to ensure that an EBV-infected naive B cell that becomes activated and starts to proliferate will rapidly exit the cell cycle and become a resting memory cell expressing none of the dangerous growth-promoting genes. In addition, the virus has conserved the targets for CTLs to ensure that if a newly infected cell does not exit the cell cycle, it will be rapidly killed.

CONCLUSIONS AND FUTURE DIRECTIONS

We now know the basic outlines of the EBV life cycle and have some understanding of where and why the tumors arise. For the basic scientist, the challenge remains to understand, at the molecular level, how EBV negotiates the changes between the different latency states in the different B-cell types. Because this is so dependent on B cells, it is likely that the mechanisms will only become clear when we learn how the processes are normally regulated in B cells. There is still also much to be learned about the role EBV plays at the molecular level in

tumorigenesis, particularly for BL. But perhaps the biggest gap in our knowledge is understanding what is different in the disruption of the immune response that leads to immunoblastic lymphoma in some cases, HD in others, and BL in yet others. Could timely immunologic intervention reduce the risk of subsequent development of these diseases?

The identification of EBV within tumors provides a potentially unique opportunity to develop tumor-specific therapy targeted at the virus that will not hurt normal cells. A good example of this is recent work showing that *in vitro* expanded, EBV-specific CTLs can be effective therapy against PTLD,[85] although they hold less promise for treatment of HD and NPC. An important and potentially fruitful area of clinical investigation will be the development of drugs specifically targeted against EBV. The best candidate latent protein might well be EBNA1, which allows replication of the viral DNA and therefore is essential for retention of the viral DNA in a proliferating (eg, tumor) cell. The crystal structure of EBNA1 bound to DNA is known, opening the path to the development of drugs that block this interaction. If the tumor requires EBV to grow, loss of the viral DNA should prevent tumor growth. Whether EBV truly plays a causative role in these tumors is therefore not an esoteric question. If the virus is not a key player, then therapies directed at the virus will be ineffective against the tumors. A hint of this comes from PTLD, in which restoration of the immune response leads to tumor regression. Eventually, however, the tumors become resistant. This raises the possibility that ultimately tumor growth might not be dependent on the virus.

An interesting approach that does not require the virus to be essential for tumor growth would be the development of drugs that efficiently cause EBV in the tumors to begin replicating. Because replication of the virus kills the cell, this would be an indirect way to destroy EBV-positive tumors, irrespective of their dependence on the virus for growth. This would not need to lead to wholesale production of virus, however, because drugs that block this are already available (eg, valacyclovir).

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