Seminars in Cancer Biology 34 (2015) 58–69

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

Cross-talk between Epstein-Barr virus and microenvironment in the pathogenesis of lymphomas

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ARTICLE INFO

Keywords:
Epstein-Barr virus
Lymphoma
Microenvironment
Exosome
Immune response

ABSTRACT

Epstein-Bar virus (EBV) is known to directly drive the neoplastic transformation of lymphoid cells resulting in the development of a variety of lymphoproliferative disorders. Emerging evidence however indicates that this final outcome is also related to the ability of EBV to shape microenvironment making it more conducive to cell transformation. Indeed, EBV up-regulates the production of several soluble factors promoting the growth and/or the survival of lymphoid cells and orchestrates a variety of complex mechanisms favoring their escape from anti-tumor immune responses. Furthermore, EBV-infected B lymphocytes actively secrete exosomes and recent investigation is now shedding light on the content and functional impact that these bioactive vesicles may have in bystander recipient cells. The complex interplay existing between EBV-carrying lymphoid cells and tumor microenvironment is now offering attractive targets of therapy that can be exploited to improve current therapeutic strategies for EBV-driven lymphoid malignancies.

1. Introduction

It is increasingly recognized that the microenvironment plays an active role in the development of lymphomas and we are only beginning to understand the complex cross talk occurring between lymphocytes and stromal cells/components during the various phases of lymphomagenesis and malignant progression of lymphoid malignancies. These interactions may critically regulate lymphoma cell homing, growth, survival, response to host immunity and therapy, and resistance to therapeutic agents [1,2]. This holds true also for lymphomas pathogenically associated with infection by oncogenic viruses, particularly Epstein-Barr virus (EBV), an ubiquitous herpesvirus usually establishing a harmless coexistence with the host but that may also directly promote the development of a variety of lymphoproliferative disorders, including Burkitt lymphoma (BL), Hodgkin lymphoma (HL), post-transplant lymphoproliferative disorders (PTLD), diffuse large-B-cell lymphomas of the elderly, AIDS-associated lymphomas, and NK/T-cell lymphomas [3,4]. Available evidence indicates that the interactions occurring between EBV and host microenvironment are relevant not only for the establishment of EBV latent infection in B cells, but also for the development of EBV-driven tumors.

In asymptomatically infected individuals, EBV resides mainly in resting, long-lived memory B lymphocytes where the virus may be completely silent in terms of expression of viral proteins (the so-called latency 0) to avoid immune recognition. How EBV reaches this cellular reservoir is still debated, although critical interactions with the microenvironment of lymphoid tissues probably play a relevant role in this process. One model proposes that EBV infection of naïve B lymphocytes drives their proliferation and expansion promoted by the concerted action of the full spectrum of EBV-encoded latency proteins, including six EBV nuclear antigens (EBNAs) and three latent membrane proteins (LMP-1, LMP-2A, LMP-2B), the so-called latency III program. A similar pattern of viral gene expression is observed in EBV+ lymphoblastoid cell lines (LCLs) obtained by EBV-mediated transformation of primary B lymphocytes *in vitro*. A fraction of EBV-infected B-cells may enter the germinal centers of lymph nodes where they express a more restricted number of viral proteins, limited to EBNA-1, LMP-1, and LMP-2 (latency II). In this particular microenvironment, where B cells physiologically undergo the process of antigen selection and affinity maturation of their immunoglobulin, EBV-infected B lymphocytes may escape apoptosis thanks to the survival signals provided by LMP-1 and LMP-2, which are functional homologues of CD40 and B-cell receptor, respectively [5]. These cells then exit from germinal center as memory B cells, usually with a latency 0 or latency 1 (EBNA-1-only) phenotype. An additional program may be activated by

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http://dx.doi.org/10.1016/j.semcancer.2015.04.006
1044-579X/

EBV in B lymphocytes terminally differentiated into antibody-secreting plasma cells, in which the virus may actively replicate through lytic cycle induction. Nevertheless, expansion of EBV+ B cells within germinal centers is only rarely detected in immunocompetent patients, being more commonly observed in the setting of immune deficiency, particularly in patients with HIV infection or with late-onset EBV+ B-cell lymphomas associated with age-related decline in EBV-specific immunity [6]. Moreover, EBV has been also found in the so-called "non-switched" memory B cells in patients with genetically determined immune deficiencies such as X-linked lymphoproliferative disease, conditions in which the formation of conventional germinal centers and the ability to mount isotype-switched antibody responses are impaired [7,8]. These findings support the hypothesis that EBV may directly infect pre-existing memory B cells, obviating thus the need of EBV-carrying naïve B lymphocytes to transit through germinal center reaction.

The EBV genome has been detected in a variety of lymphoid malignancies [4,9] in which the virus expresses different latency patterns that vary according to the activation and differentiation stage of lymphoma cells [10]. The broad latency III is mainly detected in lymphomas associated with immune deficiency, such as PTLDs and AIDS-associated immunoblastic lymphomas, whereas classical HL, nasal NK/T-cell lymphomas express only EBNA-1, LMP-1 and LMP-2 (latency II). A more restricted form of EBV latency is characteristic of BL in which EBNA-1 is the only viral protein expressed [3,4]. It should be considered, however, that the patterns of EBV latency observed in EBV-driven lymphomas may show a certain degree of intratumor heterogeneity, as demonstrated by the detection of all types of latency in EBV+ PTLDs and by the variable latency II/III observed in EBV-associated diffuse large B-cell lymphomas of the elderly [4]. As discussed below, this heterogeneity is probably mainly dependent on a different pressure of host immune responses to EBV, although microenvironmental factors able to modulate the expression of EBV latency genes may be also involved. In all EBV-associated tumors, irrespective of the different forms of latency, EBV expresses small non-polyadenylated, non-coding double-strand RNAs, the (EBV)-encoded small RNAs (EBERs), which may also promote EBV-driven B-cell immortalization [11]. EBV also encodes two different families of microRNAs (miRNAs): BART (BamHI A rightward transcript) and BHRF1 (Bam HI fragment H rightward open reading frame 1). Similarly to EBERs, BART miRNAs are expressed in all EBV latency types, whereas the expression of BHRF-1 miRNAs seems to be specific of latency type III [12–14].

Experiments performed with recombinant EBV strains lacking individual latent genes demonstrated that EBNA-2 and LMP-1 are strictly required for EBV-mediated B-cell transformation, whereas EBNA-1, EBNA-3, -5, and -6 were shown to contribute to this effect, although they are dispensable [3,4]. Full immortalization is achieved through the concerted action of several EBV proteins able to hijack and derange cellular pathways regulating growth and/or survival. LMP-1 is considered the major EBV oncoprotein, acting as an oncogene in rodent fibroblast cells [15]. LMP-1 functions as a constitutively active tumor necrosis factor receptor, mimicking an activated CD40 receptor, although structurally different [16–18]. LMP-1 has pleiotropic functions being able to promote B-cell activation, homotypic and heterotypic cell adhesion and the expression of cell surface (*i.e.* CD23, CD39, CD40, and CD44) and adhesion (LFA1, ICAM1, and LFA3) molecules [3]. LMP-1 is also responsible for the up-regulation of anti-apoptotic proteins, and may suppress cellular senescence [3]. Particularly relevant from a lymphomagenic point of view is the ability of LMP-1 to activate multiple cellular signaling pathways, including mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), phosphatidylinositol 3-kinase (PI3K)/Akt, and NF-κB [3,4]. The LMP-2A protein, by mimicking signals derived from the B-cell receptor (BCR), may provide a constitutive stimulus allowing the survival of EBV-infected B lymphocytes, regardless of the expression of a competent BCR or the presence of appropriate antigenic stimulation [19]. Moreover, LMP-2A also blocks signaling through the BCR that would lead to lytic reactivation [20]. EBNA-1 is a DNA-binding nuclear protein playing critical roles in replicating and maintaining the EBV episomes as well as in transactivating its own expression and that of other EBV latency genes important for cell immortalization [21]. EBNA-1 was also shown to enhance cell survival, genomic instability and DNA damage responses, and it may induce the expression of the RAG1 and RAG2 recombinases [21]. The EBNA-2 nuclear protein is one of the first viral proteins expressed in EBV-infected B-lymphocytes and, together with EBNA-5, promotes the G_0 to G_1 transition of resting B cells and strongly up-regulates LMP-1 and LMP-2 expression [22]. In addition, EBNA-2 modulates the transcriptional activity of different B-cell activation markers including CD21, CD23, hes-1, runx3, and the proto-oncogene c-MYC [22]. The function of EBNA-2 as transcriptional transactivator mainly depends on its ability to bind RBP-Jκ, thus mimicking a constitutively activated Notch receptor, which is frequently activated in lymphomas [23]. Studies carried out with viral miRNA deletion mutants indicate that EBV miRNAs contribute to EBV-driven B-cell immortalization *in vitro*, being however not essential [4]. For a more detailed description of the functional properties of the various EBV-encoded RNAs and proteins refer to specific reviews [3,24,25].

The initial concept that only the latency phase of EBV infection is relevant for the development of EBV-associated lymphomas has been recently challenged by evidence indicating that lytic EBV replication may also have a critical pathogenic role. Small numbers of lytically infected cells are in fact frequently detected in pathologic tissues of EBV-associated lymphomas [26]. Besides favoring the local and systemic spread of the virus, lytic infection may increase the pool of latently infected cells, which is associated with a higher risk of a clonal expansion of EBV-carrying B lymphocytes in the immune compromised host. Intriguingly, LCLs generated with lytic-defective EBV strains were shown to be markedly less effective in the induction of EBV+ lymphoproliferations in SCID mice, an effect related to the lower production of the B-cell growth promoting factors [27,28].

A growing body of evidence indicates that microenvironmental factors may influence all phases of the complex interactions between EBV and infected host occurring in both health and disease. The present review is aimed at rationally summarizing available data concerning the complex interplay that EBV-infected B cells may have with the network of non-neoplastic immune and stromal cells/components with a particular focus on the impact of these interactions on EBV-driven lymphomagenesis.

2. EBV-mediated local production of factors promoting lymphoid cell growth and survival

The EBV-encoded proteins expressed in the different EBV-driven B-cell lymphoproliferations are able to up-regulate the production of several soluble factors promoting the growth and/or the survival of lymphoid cells. PTLDs include a broad spectrum of heterogeneous lymphoproliferative diseases that may occur in the setting of acquired immune deficiency after allogeneic transplantation. Most of the cases, particularly those occurring early after transplantation, are associated with EBV and are mainly characterized by the broad type III latency expression pattern [29], similarly to *in vitro* grown EBV-immortalized LCLs. In this setting, the lack of effective anti-EBV immune responses allows EBV to fully exploit all its capabilities to activate a wide array of cellular signaling pathways that may enhance the production of B-cell growth-promoting factors and increase resistance to apoptosis (Fig. 1). Moreover, EBV