
Preface

Fifty-two years ago, collaboration between a dedicated surgeon and basic researchers, who applied a combination of as diverse laboratory methods as in vitro cell culture and electron microscopy, paved the way to the discovery of Epstein–Barr virus (EBV), a human herpesvirus (reviewed by Epstein [1]). The detection of herpesvirus particles in Burkitt lymphoma (BL) cells supported the idea—suggested by Denis Burkitt—that an infectious agent may cause BL, a B cell lymphoma highly prevalent among children in Equatorial Africa. Ever since, newer and newer analytic methods were applied to the field of EBV research, shaping our current views as to the natural history of EBV and the pathogenesis of EBV-associated diseases (for a historical perspective, see Ref. [2]). Furthermore, BL was regarded “as a Rosetta stone for understanding the multistep carcinogenesis,” i.e., observations such as EBV and *Plasmodium malariae* infection of children with endemic BL and the discovery of *c-myc* translocations in BL cells influenced thinking as to the development of other neoplasms, too [3]. De Thé et al. referred in their paper to a bilingual text, carved in three scripts into a rock stele that was found in the Nile Delta near the town of Rosetta (Rashid); that text—a new research tool, if you wish—was instrumental for the decipherment of Egyptian hieroglyphs by Jean-François Champollion, an achievement that allowed the translation of Ancient Egyptian texts and facilitated the understanding of Egyptian culture and civilization [4]. The data gained by the application of molecular biological methods combined with the applications of up-to-date immunological and cytogenetic methods as well as in vitro and in vivo models of EBV research may offer new solutions to unresolved problems of the EBV field. Some of the hot topics and alternative scenarios as to the viral life cycle, latency, and EBV-associated diseases are overviewed in Chapter 1 of this volume, in the light of the most recent findings, by Janos Minarovits and Hans Helmut Niller. This introductory chapter is followed by typical *Methods* chapters written by experts of the respective areas. Hans Helmut Niller and Georg Bauer give a description of the immunological and molecular methods used in EBV diagnostics (Chapter 2). It is not easy to investigate in vivo EBV infection of B lymphocytes and epithelial cells, the major cell types targeted by EBV. The use of in vitro models facilitates, however, the study of EBV–host cell interactions. Such models include the establishment of “immortalized” lymphoblastoid cell lines by EBV infection of human B lymphocytes, as presented by Noémi Nagy (Chapter 3), and the use of organotypic cultures for the analysis of EBV–epithelial cell interactions, as described thoroughly by Rachel M. Temple, Craig Meyers, and Clare E. Sample (Chapter 4). Identification of the interacting viral and cellular proteins is indispensable for the understanding of interrelationships between EBV and its host cells. In Chapter 5, Anna A. Georges and Lori Frappier guide us how to use affinity purification-mass spectroscopy methods for identifying protein–protein interactions in EBV-infected cells. Certain latent EBV proteins influence the nuclear architecture of host cells. Hans Knecht and Sabine May elaborated a spectacular method for the characterization—in three dimensions—of nuclear architecture. They demonstrate how to use 3D Telomere FISH (fluorescent in situ hybridization) to detect nuclear changes, including the alterations of chromosomal ends, induced by LMP1 (latent membrane protein

1, a viral oncoprotein) in Hodgkin lymphoma cells (Chapter 6). Next-generation sequencing methods allow the detailed analysis of transcript structure and abundance in EBV-infected cells. In Chapter 7, Tina O'Grady, Melody Baddoo, and Erik K. Flemington outline the use of high-throughput RNA sequencing for the analysis of EBV transcription and guide the reader regarding the application of informatics tools for the analysis and visualization of sequence data. Two other approaches, quantitative polymerase chain reaction (qPCR) following conventional RNA isolation and nuclear run-on assay that are suitable for the study of viral promoter activity, are described in Chapter 8 by Kálmán Szenthe and Ferenc Bánáti. In addition to mRNAs, the EBV genome also encodes nontranslated viral microRNAs that modulate the level of both viral and cellular mRNAs and proteins. Furthermore, EBV latency products may alter the level of cellular microRNAs as well. Accordingly, the analysis of viral and cellular microRNAs, the topic of Chapter 9 written by Rebecca L. Skalsky, is of primary importance in the characterization of EBV-infected cells. MicroRNAs and other biomolecules are packaged into exosomes, small lipid vesicles involved in intercellular communication. EBV-infected cells use exosomes for information transfer as well, and the methods for exosome isolation and characterization as well as their functional analysis are detailed in Chapters 10 and 11 by Gulfaraz Khan and Waqar Ahmed, and Gulfaraz Khan and Pretty S. Philip, respectively. Regarding the methods used to study viral DNA, in Chapter 12 Ferenc Bánáti, Anita Koroknai, and Kálmán Szenthe describe how the clonality of a cell population carrying latent EBV episomes can be inferred from the "classical" terminal repeat analysis of the viral genome, whereas Chapter 13, by Kálmán Szenthe and Ferenc Bánáti, deals with the application of sequencing for the characterization of viral promoters and coding regions. Similarly to cellular promoters, the activity of latent and lytic EBV promoters is also regulated by the binding of viral and cellular regulatory proteins and by epigenetic mechanisms. In Chapter 14, Anja Godfrey, Sharada Ramasubramanian, and Alison J. Sinclair demonstrate the use of ChIP-Seq method (chromatin precipitation coupled to DNA sequencing) for the analysis of Zta-DNA interactions. Zta, also called ZEBRA, is an immediate early protein switching on lytic (productive) EBV replication, and there are Zta binding sites both in the viral and in the cellular genome. Host cell phenotype-dependent deposition of epigenetic marks, including DNA methylation and histone modifications, determines the epigenotypes of latent EBV episomes and the activity of the latency promoters. In a detailed protocol, Daniel Salamon describes the use of bisulfite sequencing for the analysis of cytosine methylation in EBV DNA sequences and lists thoroughly the important steps and caveats of bisulfite modification and PCR amplification (Chapter 15), whereas in Chapter 16 Ferenc Bánáti and Kálmán Szenthe outline how chromatin immunoprecipitation using specific antibodies directed to distinct histone modifications can be applied for the characterization of viral epigenotypes. In vivo experimental models may help to gain insight into important aspects of the EBV life cycle and into the pathogenesis of EBV-associated diseases that are difficult to study in EBV-infected humans. In Chapter 17, Frank Heuts and Noemi Nagy describe how newborn immunodeficient mice transplanted with human hematopoietic stem cells can be used for the study of immune interactions that occur during EBV infection, whereas in Chapter 18 Ken-Ichi Imadome and Shigeyoshi Fujiwara provide detailed protocols for the preparation and EBV infection of humanized mice and for the monitoring of virological and immunological consequences of the infection. They also describe the development of EBV-associated lymphoproliferative disease in such mice.

Finally, Lauren P. McLaughlin, Stephen Gottschalk, Cliona M. Rooney, and Catherine M. Bollard describe, in Chapter 19, how immunological, virological, tissue culture, and molecular methods can be combined to yield GMP (Good Manufacturing Practice)-compliant EBV-specific T cells for the immunotherapy of EBV-associated post-transplant lymphoproliferative disease (PTLD).

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