Mononucleosis in the Laboratory

Richard F. Ambinder and Lan Lin
Department of Oncology, Johns Hopkins School of Medicine, Baltimore, Maryland

(See the articles by Balfour et al. and Woodberry et al., on pages 1505–12 and 1513–24, respectively.)

Laboratory investigation of infectious mononucleosis long preceded any knowledge of the etiologic virus [1]. The characteristic mononuclear leukocytosis associated with Pfeiffer glandular fever was reported in 1920. Heterophile antibodies were recognized 12 years later [2]. A specific viral link was established only after the serendipitous observation that a technician recovering from infectious mononucleosis had seroconverted to Epstein-Barr virus (EBV) [3]. A series of studies shortly thereafter confirmed the association.

In more recent years, a great deal of effort has focused on the viral genome, structure and function of the virion, and aspects of gene regulation, particularly lymphocyte-immortalizing and growth-transforming properties and association with various types of tumors—including Burkitt lymphoma, nasopharyngeal carcinoma, posttransplantation lymphoma, Hodgkin lymphoma, AIDS lymphoma, nasal lymphoma, leiomyosarcoma in immunocompromised patients, and gastric carcinoma [4]. In this issue of the Journal of Infectious Diseases, 2 investigations of the laboratory correlates of infectious mononucleosis are presented. One is focused on measurement of viral copy number [5], and the other is focused on the cellular immune response to viral antigens [6].

In considering the study by Balfour et al. [5] of viral copy number in association with infectious mononucleosis, it must be remembered that measurements of viral copy number may reflect the presence of latent or lytic infection or both (figure 1). Thus, latently infected B cells harbor double-stranded viral episomes, and the measurement of viral copy number in blood may simply reflect the number of latent episomes in B cells. But B cells may also support lytic cycle replication, and viral copy number may therefore reflect virion production. In cell-free blood (serum or plasma), encapsidated viral genomes (virions) may be detected. But viral DNA may be also be released without the protective virion capsid or envelope from latently infected cells, most notably tumor cells undergoing apoptosis.

In patients with nasopharyngeal carcinoma, EBV DNA is detected in high copy numbers in serum or plasma but not in mononuclear cells [7–11]. Pretreatment viral copy number in cell-free blood is an important adverse prognostic factor, as is the persistence or recrudescence of high copy numbers of viral DNA in serum or plasma. Evidence that the viral DNA in serum or plasma is predominantly tumor derived includes its rapid disappearance after surgical excision of the tumor (a rare procedure) and its somewhat slower disappearance, after a transient increase, with radiation therapy (a standard approach). Sensitivity to DNase digestion suggests that the DNA is not encapsidated, and its fragmentation pattern suggests that it is released from cells undergoing apoptosis. The situation in nasal lymphoma, gastric carcinoma, and Hodgkin disease appears to be similar.

In posttransplantation lymphoproliferative disease, viral DNA is also detected in high copy numbers in peripheral blood mononuclear cells (PBMCs) and in cell-free blood [11–13]. Viral copy numbers in PBMCs are accounted for by a large increase in the number of latently infected
resting B cells and, perhaps, an increase in the number of lytically infected B cells. Neither of these populations of circulating cells necessarily includes tumor cells. In HIV-infected patients, the EBV copy number in PBMCs increases rapidly after HIV infection, and that increase precedes any decrease in the CD4 T cell count. Viral copy number in PBMCs has not been shown to be predictive of the development of lymphoma [14].

For all that is known about virus copy number in these other settings, the simplest questions regarding symptomatic infection have not been systematically addressed until now. The article by Balfour et al. extends the study of viral copy number to patients with infectious mononucleosis, in whom viral copy number in whole blood correlates with the severity and duration of symptoms but viral copy number in oral wash fluid seems to be independent of symptoms. Although these findings represent an important, if expected, advance, the limitations of such studies are significant. They do not, however, address the question of whether the higher copy number in the most symptomatic patients reflects predominately viremia (virions in cell-free blood) or predominantly latently infected proliferating B lymphocytes or a composite of virions in cell-free blood and viral DNA in latently infected lymphocytes. The observation that there is little relation between oral shedding and severity of symptoms highlights the limitations of our knowledge about oral shedding. Indeed, whether the symptoms of infectious mononucleosis, such as pharyngitis, are the result of viral lysis of oropharyngeal epithelial cells, an inflammatory response to viral infection, or both is not clear. Similarly, whether the hepatitis that commonly accompanies infectious mononucleosis is caused by an infection of hepatocytes or misguided immune responses is unknown.

The immune response during infectious mononucleosis is characterized by the presence of activated lymphocytes (primarily CD8⁺ cytotoxic T lymphocytes). Although the possibility that this expansion might be the result of a superantigen-driven process has been entertained, analysis of the diversity of antigen receptors favors the idea that these T cells are antigen specific [15]. Woodberry et al. [6] present what is to date the most comprehensive study of the evolution of the cellular immune response over time and comparisons of the response in HLA-matched siblings with and without a history of infectious mononucleosis. Their results point to no sustained difference in the cellular immune response that accompanies symptomatic primary EBV infection, although, as they acknowledge, there is much more work to be done.

References