CEBP Focus: Update on Lymphoma

Infectious Agents as Causes of Non-Hodgkin Lymphoma

Eric A. Engels

Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Department of Health and Human Services, Rockville, Maryland

Abstract

Among exposures presently viewed as possible etiologic factors in non-Hodgkin lymphoma (NHL), infections are close to being regarded as established causes. Infectious agents causing NHL can be classified, according to mechanism, into three broad groups. First, some viruses can directly transform lymphocytes. Lymphocyte-transforming viruses include Epstein Barr virus (linked to Burkitt's lymphoma, NHLs in immunosuppressed individuals, and extranodal natural killer/T-cell NHL), human herpesvirus 8 (primary effusion lymphoma), and human T lymphotropic virus type I (adult T-cell leukemia/lymphoma). Second, human immunodeficiency virus is unique in causing profound depletion of $CD4^+$ T lymphocytes, leading to acquired immunodeficiency syndrome and an associated high risk for some NHL subtypes. Third, recent evidence suggests that some infections increase NHL risk through chronic immune stimulation. These infections include hepatitis C virus as well as certain bacteria that cause chronic site-specific inflammation and seem to increase risk for localized mucosa-associated lymphoid tissue NHLs. Establishing that an infectious agent causes NHL depends on showing that the agent is present in persons with NHL as well as laboratory experiments elucidating the mechanisms involved. Only epidemiologic studies can provide evidence that infection is actually a risk factor by showing that infection is more frequent in NHL cases than in controls. Given the range of mechanisms by which infections could plausibly cause NHL and our growing molecular understanding of this malignancy, this field of research deserves continued attention. (Cancer Epidemiol Biomarkers Prev 2007;16(3):401–4)

Among the exposures presently viewed as possible etiologic factors in the development of non–Hodgkin lymphoma (NHL), infections are perhaps closest to being regarded as established causes. Indeed, immunosuppression, another strongly implicated NHL risk factor, may act, in part, to increase the cancer-causing effects of specific infectious agents. Just as there are only a few infectious agents that seem capable of causing NHL, NHL itself is heterogeneous, and not all NHL subtypes are etiologically related to infection. This review presents a classification of mechanisms by which infections cause NHL, considers evidence linking specific infections with NHL, and suggests avenues for future research.

Known or suspected infectious causes of NHL can be classified, according to mechanism, into three broad groups (Table 1). First, certain lymphocyte-transforming viruses can directly infect lymphocytes, disrupt normal cell functions, and promote cell division. Second, human immunodeficiency virus (HIV) infection is unique in causing profound depletion of $CD4^+$ T lymphocytes, leading to acquired immunodeficiency syndrome (AIDS). AIDS is associated with a markedly elevated risk of high-grade B lymphocyte NHLs. Third, some infections seem to increase NHL risk by inducing chronic immune stimulation and persistent activation of lymphocytes. Two additional hypothesized mechanisms are not included within the framework presented in Table 1 because supporting evidence is weaker: the ''hit-and-run'' hypothesis, which posits that an infectious agent critically damages a lymphocyte but is no longer present in the host at the time of NHL development, and the ''hygiene hypothesis,'' which posits that the timing of exposure to common infectious agents, in early childhood, modulates NHL risk later in life.

Epstein Barr virus (EBV) is an important example of a transforming virus implicated in several NHL subtypes (1). EBV is usually acquired in early childhood in developing countries. In developed countries, primary infection in adolescence is associated with the clinical syndrome infectious mononucleosis. Following primary infection, EBV persists lifelong in the host in a latent state in memory B lymphocytes. EBV readily transforms B lymphocytes *in vitro*. Multiple EBV proteins can be expressed in infected lymphocytes, among which latent membrane protein-1 is thought to be most important for transformation. In healthy infected individuals, outgrowth of EBV-transformed B lymphocytes is prevented by the presence of intact T lymphocyte-mediated immunity (2).

Dating from the initial discovery of EBV in Burkitt's lymphoma cells, EBV has consistently been linked to this NHL subtype. Incidence of this subtype is especially high in sub-Saharan Africa, where the tumor typically presents in young children as a rapidly growing extranodal mass (''endemic'' Burkitt's lymphoma). Burkitt's tumor cells characteristically show a t(8;14) translocation activating the c-*myc* oncogene. The nearly universal presence of EBV DNA and proteins in tumor cells implicates EBV in endemic Burkitt's lymphoma. Further supporting an etiologic role for EBV, African children who develop Burkitt's lymphoma manifest elevated antibody titers against the EBV capsid several years before diagnosis (3). In addition, EBV is itself monoclonal in tumor cells, suggesting initial infection of a single precursor cell as an early tumor-inducing event (4). Nonetheless, EBV-infected Burkitt's tumor cells do not express latent membrane protein-1. Consequently, the molecular mechanisms for EBV-mediated transformation are not well understood (2). Based on the geographic distribution of endemic Burkitt's lymphoma, malaria caused by *Plasmodium falciparum* is also postulated to play a key role in the development of this tumor, perhaps by

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Requests for reprints: Eric A. Engels, Viral Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Department of Health and Human Services, 6120 Executive Boulevard, EPS 7076, Rockville, MD 20852. E-mail: engelse@exchange.nih.gov

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causing chronic immune stimulation or immunosuppression (Table 1; ref. 5).

EBV is also strongly implicated in other NHLs arising in congenital or acquired immunodeficiency states. In organ transplant recipients, iatrogenic immunosuppression leads to loss of control of EBV infection and a spectrum of EBV-driven lymphoproliferation, ranging from hyperplasia to frank NHL (6). Primary EBV infection occurring after transplant is associated with marked NHL risk. Transplant-associated NHLs, when EBV positive, express a broad range of EBV proteins, including latent membrane protein-1. In addition, EBV is almost always found in cases of extranodal natural killer/T-cell NHL (7), a rare NHL subtype that is usually localized in the nasal sinuses (also known as ''lethal midline granuloma'').

Two other lymphocyte-transforming viruses, human herpesvirus 8 (HHV8) and human T lymphotropic virus type I (HTLV-I), deserve mention (Table 1). HHV8, the causative agent of Kaposi's sarcoma, is evolutionarily related to EBV. HHV8 is found in all cases of primary effusion lymphoma, a rare variant arising in serous body cavities (8). HHV8 is also closely linked with multicentric Castleman's disease and the plasmablastic NHLs that occur in association with this disorder (9). HTLV-I is the established cause of adult T-cell leukemia/lymphoma (10).

HIV causes NHL by inducing profound cell-mediated immunodeficiency, which permits dysregulated proliferation of B lymphocytes. EBV is often involved in AIDS-associated NHLs. Three NHLs, with varying rates of EBV positivity, are characteristic of AIDS: central nervous system NHL (almost universally EBV positive), diffuse large B-cell lymphoma (DLBCL; ~50% EBV positive), and Burkitt's lymphoma (~30% EBV positive; ref. 1). In central nervous system NHL and DLBCL, tumor cells usually express EBV latent membrane protein-1 (11). People with AIDS have markedly elevated risk for these NHL subtypes, with standardized incidence ratios of 5,000 for central nervous system NHL, 100 to 140 for DLBCL, and 60 for Burkitt's lymphoma (12). In AIDS, risk for central nervous system NHL and DLBCL increases linearly as the CD4 lymphocyte count declines (13). The atypical pattern in AIDS-associated Burkitt's lymphoma (less frequent EBV positivity, lower risk relative to the general population, lack of relationship with CD4 count) suggests that immunosuppression is less directly involved than in other AIDS NHL. HIV infection is also associated with chronic B lymphocyte activation, which could be etiologically relevant for Burkitt's or other NHL subtypes. In addition, although primary effusion lymphoma is uncommon even among people with AIDS, risk for this subtype is increased compared with the general population, highlighting the importance of HIV-induced immunosuppression in permitting HHV8-driven lymphoproliferation. Among people with AIDS, HHV8 is also found in a subset of immunoblastic or anaplastic DLBCLs histologically related to primary effusion lymphoma (14, 15).

Table 1. Infections associated with NHL

Infectious agent	NHL subtype
Lymphocyte-transforming viruses	
Esptein Barr virus	Burkitt's lymphoma
	AIDS-associated NHLs
	Posttransplant lymphoproliferative disorder
	Extranodal natural killer/T-cell NHL
Human Herpesvirus 8	Primary effusion lymphoma and related DLBCL
	MCD-associated plasmablastic NHL
Human T lymphotropic virus type I	Adult T-cell leukemia/lymphoma
Agents that cause immunosuppression	
Human Immunodeficiency Virus	AIDS-associated NHLs
Agents that cause chronic immune stimulation	
*Plasmodium falciparum**	Burkitt's lymphoma
Hepatitis C virus	Uncertain
Helicobacter pylori	Gastric MALT NHL
Campylobacter jejuni	Small intestine MALT NHL
Chlamydia psittaci	Ocular adnexa MALT NHL
Borrelia afzelii	Cutaneous MALT NHL

Abbreviation: MCD, multicentric Castleman's disease; AIDS, acquired immunodeficiency syndrome; DLBCL, diffuse large B cell lymphoma; MALT, mucosa-associated lymphoid tissue.

*The possible mechanism of action of *P. falciparum* malaria in causing Burkitt's lymphoma is not well characterized and may involve immunosuppression.

Hepatitis C virus (HCV) is an example of an infectious agent implicated in causing NHL through chronic immune stimulation. Following primary infection, HCV usually produces chronic hepatitis and viremia. Persistent HCV infection is associated with a range of immune-related conditions, including essential mixed cryoglobulinemia, a low-grade lymphoproliferative disorder that can progress to NHL (16). Although HCV may be able to infect lymphocytes, a direct transforming role has not been shown. Instead, lymphoproliferation may be due to binding of HCV to receptors on the surface of B lymphocytes (17), which could lower their threshold for antigen response or induce DNA mutations. HCV-infected individuals may also have an increased prevalence of circulating lymphocytes with abnormal chromosomal translocations associated with NHL [e.g., t(14;18) (18)].

The association between NHL and HCV infection has been examined in many retrospective case-control studies (19). Most of these studies, although not all, have found a positive association, with odds ratios ranging from 2 to 10. Notably, there is substantial heterogeneity in the magnitude of association across studies (19), and it has been difficult to determine the degree to which this heterogeneity can be attributed to demographic differences in study populations or, instead, methodologic differences in the studies (e.g., choice of control subjects). Whether HCV is associated with an increased risk for all NHL subtypes or only certain subtypes is an important unresolved question. Among HCV-infected patients with splenic marginal zone NHL, treatment of infection with interferon-α–based regimens can lead to HCV clearance and, simultaneously, regression of NHL (20). This observation implies that HCV infection is causal in at least a subset of NHLs, directly driving lymphoproliferation. HCV coinfection is common in HIV-infected individuals, but HCV does not increase risk for AIDS-associated NHLs (21).

Mucosa-associated lymphoid tissue (MALT) NHLs arise in aggregates of lymphocytes associated with sites of chronic inflammation (22, 23). Histologically, these NHLs consist of postgerminal center lymphocytes and resemble marginal zone NHLs. MALT NHLs can develop in association with autoimmune conditions involving site-specific inflammation. Thus, Sjögren's syndrome (an autoimmune condition characterized by chronic salivary gland inflammation) is associated with a strongly increased risk of salivary gland MALT NHL, and autoimmune thyroiditis is associated with thyroid MALT NHL. Notably, there is an emerging body of evidence linking certain bacteria that cause chronic inflammation with MALT NHLs at those sites (Table 1). The strongest data associate gastric MALT NHL with chronic gastritis caused by *Helicobacter pylori*. Seroprevalence of *H. pylori* is higher among individuals with gastric MALT NHL than among controls, and in many cases, antibacterial therapy directed at this bacterium can lead to regression and remission of the associated tumor (23).

Table 2. Evidence supporting an etiologic association between an infectious agent and NHL

Evidence	Comment
Detection of agent in persons with NHL	Highly sensitive techniques provide important clues, but false-positive results occur. Confirmation of detection with multiple methods in several laboratories is necessary
Detection of agent in tumor cells	
Detection of host infection (e.g., antibody)	
Experiments on mode of action	Experimental models can provide strong support for a proposed etiologic relationship and suggest markers for studies in humans
Animal models	
In vitro infection of lymphocytes and transformation	
Molecular and cellular biology data on intracellular pathways	
Epidemiologic studies	Epidemiologic studies in humans are required to estimate the magnitude of association between the infectious agent and NHL
Show that infection is more common in NHL cases than in controls	
Show that infection precedes NHL	
Specificity of association	The association of one agent with only one or a few NHL subtypes supports an etiologic relationship but is not required

More recently, several studies have linked three other site-specific MALT NHLs with bacteria causing chronic local infections: small intestine NHL with *Campylobacter jejuni*, ocular adnexa NHL with *Chlamydia psittaci*, and cutaneous NHL with *Borrelia afzelii* (a European agent of Lyme's disease; refs. 24-26). These initial observations await confirmation and extension, although the work is made difficult by the rarity of these NHLs.

It is useful to consider the types of data that, in general, support an etiologic association between specific infections and NHL (Table 2). First, it is important to show that infection is actually present in persons with NHL. For lymphocyte-transforming viruses, the agent can be detected within tumor cells using sensitive tests for viral DNA, RNA, or protein. For agents that act less directly, similar techniques can be used to detect host infection elsewhere (e.g., *H. pylori* infection is shown by detection of the bacterium in the stomach or detection of host antibodies to the bacterium). Because false-positive results can occur, supportive data using multiple detection methods are required. Second, the argument for etiologic association needs support from laboratory experiments that clarify the potential mechanism of action. Relevant data derive from animal models of infection-caused lymphomagenesis, experiments showing *in vitro* infection and transformation of lymphocytes, and detailed maps of implicated molecular pathways. Third, epidemiologic data are required. Only epidemiologic studies can provide clear evidence that infection is actually a risk factor for NHL by showing that infection is more frequent in NHL cases than controls. Because NHL or its treatment can alter the manifestations of infection (e.g., by altering antibody levels), retrospective case-control studies may sometimes provide inaccurate results. Thus, prospective studies that show infection precedes NHL and that infection is associated with subsequently elevated NHL risk can be especially compelling. Fourth, as we learn that NHL is a heterogeneous malignancy, in relation to molecular characteristics, treatment response, and outcome, it is useful to consider the possibility that particular infections might be causes of unique NHL subtypes. A close correspondence could occur if an agent has a specific molecular mode of action or if the effects of the agent are restricted to its site of infection (e.g., *B. afzelii* and cutaneous MALT NHL). In other cases, a close correspondence between agent and NHL subtype may not exist. Agents that broadly affect the immune system (e.g., HIV and HCV) could plausibly increase risk for many NHL subtypes. Although specificity of association between agent and NHL subtype supports an etiologic relationship, it should not be viewed as necessary. These considerations on etiologic evidence parallel those proposed by Hill (27) and, specifically for infectious agents, Fredericks and Relman (28).

Given the range of mechanisms by which infections could plausibly cause NHL and our growing molecular understanding of this malignancy, this field of research deserves continued attention. Work is needed to understand the mechanisms of action of the agents discussed in this review and to identify new infectious causes of NHL. New agents can be sought in NHLs with characteristic geographic distribution (implicating geographically restricted infections), strong association with immunosuppression (implicating lymphocyte-transforming viruses), or localization to a specific body site (implicating chronic site-specific infection). Because the immune system modulates the host response to infection, an important area for further epidemiologic investigation is the systematic evaluation of the interactions of polymorphisms in immune-related genes with specific infectious agents in causing NHL.

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