Molecular Genetic Biomarkers in Hematological Malignancies

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Abstract   Most hematopoietic malignancies are widely disseminated even in their "early" stages and often do not have a well-defined localized phase. This makes them less amenable to conventional early screening methods such as imaging and observation. Furthermore, the staging systems for lymphomas are not particularly useful prognostically, with the possible exception of Hodgkin's disease. However, as currently compared with solid tumors, the extensively detailed understanding of the acquired (somatic) genetic lesions in leukemias and lymphomas provide useful molecular biomarkers for early detection. Moreover, well described high risk groups have been identified. These include individuals who are immunosuppressed, for example, iatrogenically following organ transplantation or those with AIDS. Also at high risk are patients treated with certain chemotherapeutic agents who are at risk for the development of acute non-lymphoblastic leukemia. Accordingly, these clinical settings might prove to be good models for evaluating molecular cancer risk markers and the possible introduction of chemoprevention. Here, we outline the biological basis for the application of biomarkers for the early detection of hematological neoplasia. These concepts may provide the stage for the creation of chemoprevention studies in leukemia and lymphoma. J. Cell. Biochem. 25S:165–171. © 1997 Wiley-Liss, Inc.

Key words: biomarkers; leukemia; lymphoma; molecular genetics; risk factors

In the United States in 1995, there were predicted to be approximately 45,000 new patients with non-Hodgkin's lymphoma (3.6% of total of newly diagnosed cancers), 30,000 new patients with various forms of leukemia (2.4% of total), and approximately 7,500 patients with Hodgkin's disease (0.6% of total) [Wingo et al., 1995]. Overall, these hematologic malignancies accounted for approximately 6.6% of all newly diagnosed cancers in this country during 1995. The majority of hematopoietic malignancies present themselves clinically as widely disseminated neoplasms, even in their "early" stages, and they usually do not have a well-defined localized or incipient phase. This makes them less amenable to conventional methods for early detection, such as clinical, radiological, invasive, or pathologic observation, and palpation. Furthermore, the staging systems for the lymphomas are relatively ineffective prognostic indicators, with the exception of Hodgkin's disease. However, among all cancers, acquired or somatic genetic lesions are currently best elucidated and understood in leukemias and lymphomas [Bagg, 1995]. Accordingly, these malignancies should prove to be good models for evaluating potential cancer risk markers. Here we discuss the potential role of such genetic markers in specific groups of the population known to be at risk for the development the commonest type of hematologic malignancy, the non-Hodgkin's lymphomas. Other high-risk groups, including the secondary/chemotherapy-induced acute non-lymphoblastic leukemias [Boffetta and Kaldor, 1994; Smith et al., 1994], will not be discussed in this brief review.

Since the early 1970's there has been a greater than 50% increase in the incidence of non-Hodgkin's lymphoma, with a dramatic annual rise of 3–4% [Ries et al., 1991]. Some of this rising incidence is attributable to the HIV epidemic and iatrogenic immunosuppression in the context of organ transplantation. Other

identified risk groups include those exposed to certain environmental and occupational factors, as well as infections (other than in the setting of immunosuppression). Consequently, well identified risk groups should provide a good pool for possible early detection. Since the vast majority of NHL are of B-cell lineage, particularly in the setting of immunosuppression, a very sensitive and specific tool for early screening is polymerase chain reaction (PCR) analysis of the immunoglobulin heavy chain (IgH) gene [Sioutos, et al, 1995]. This assay allows for the sensitive detection of one clonal B-cell, when diluted within a background of a million non-neoplastic, reactive cells. The specificity of the analysis is due to the fact that any B-cell (mono)clone will harbor a unique VDJ (variable-diversity-joining) rearrangement, with the exact (and specific) PCR-band size being largely determined by the random insertion of N-nucleotides by the enzyme terminal deoxynucleotidyl transferase (Tdt).

IMMUNE DYSREGULATION

There are a number of different clinical scenarios of immune dysregulation, in which there is a significant increase in the incidence of NHL, including HIV infected patients, post-transplantation, congenital abnormalities of the immune system, and autoimmune disorders.

HIV

Patients infected with HIV have an approximately 60 times increased risk of developing NHL as compared to the uninfected population [Levine, 1992], with a cumulative incidence of 5–10% over a decade [Obrams and Grufferman, 1991]. These lymphomas often differ from those in the general population at a number of levels. In particular, they are nearly always high grade (large cell immunoblastic or small non-cleaved) and often present in extranodal sites. It has been predicted that nearly one-third of all newly diagnosed patients with NHL will be those infected with HIV [Gail et al., 1991], and this is likely to increase as HIV-infected individuals live longer due to advances in supportive care and antiretroviral therapy.

The pathogenesis of NHL in HIV is similar to that which is believed to exist in endemic Burkitt's NHL, with an analogous multistep model. Four factors are believed to underly the persistent generalized lymphadenopathy (PGL) phase of HIV infection, during which B-cell oligo-

clones emerge: decreased immune surveillance (with diminution in CD4 counts), chronic antigenic stimulation (often driven by self-antigens), EBV infection (particularly in the context of the large cell, immunoblastic form of NHL), and cytokine dysregulation (especially IL-6 and IL-10) [Gaidano and Carbone, 1995]. Accordingly, these factors could be measured, and form the basis for early monitoring. Additional genetic "hits," such as c-myc dysregualtion (due to the t[8;14] chromosomal translocation, juxtaposing this protooncogene with the transcriptionally active IgH locus) and ras mutation, are usually required prior to the development of overt malignancy, and the measurement of these could also be utilized for screening purposes. Indeed, molecular and biologic differences appear to distinguish the two forms of HIV-associated NHL (Table I), so that different genetic biomarkers may be appropriate in different scenarios.

Given the common finding of a t(8;14) in many HIV associated NHLs, the frequency of this translocation in peripheral blood lymphocytes was recently evaluated in homosexual men [Muller et al., 1995]. By PCR, c-myc-IgH translocation events were found in 10.5% of those who were HIV positive and 2.0% of HIV negative individuals. In the majority of cases, abnormalities were found at a single timepoint only. However, some individuals were noted to have multiple clones (up to 4), and in others persistent clones (up to 9 years) were present.

TABLE I. Differences between Diffuse Large Cell (DLC) and Small Non-Cleaved Cell (SNCC) NHL Occurring in HIV-Positive Individuals[1]

	DLC	SNCC
Risk factors		
CD4 count	<50/μl	>200/μl
IL-10	−	+
PGL-phase	−	+
EBV	~70%	~30%
Genetic lesions		
myc	~20%	~100%
bcl-6	~20%	~0%
p53	~0%	~60%
Clinical features		
onset	late	early
site	CNS	non-CNS
frequency	~65%	~35%
prognosis	poor	"good"

[1]See reference 10.

Some point mutations in the c-myc gene were also found in some nonpersistent clones. Importantly, no correlation between the presence of these clones and the subsequent development of NHL was shown, suggesting that further, as yet uncharacterized, genetic lesions are required to fully transform the B-cells. Nevertheless, this PCR-detectable translocation may prove to be a useful screening parameter.

Predictive factors. Attempts have been made to identify factors which would predict the development of NHL in HIV+ patients [Pluda et al., 1993], in addition to those alluded to above. The only factor which has been established as a probable predictive factor for the subsequent development of NHL is the blood CD4 count, whereby those patients whose CD4 count drops below 50/μl are at increased risk for the development of NHL. A possible association between elevated serum IL6 levels and the development of NHL was not found to be statistically significant. However, this is interesting mechanistically, in that IL6 may function to rescue clones from apoptosis, since c-myc dysregulation is toxic to cells which are not protected by the presence of certain cytokines [Evan et al., 1992]. Different forms of anti-HIV infection therapy (AZT vs. DDI) appear to have no affect on the development of NHL [Pluda et al., 1993].

Post-Transplantation Lymphoproliferative Disorders

A heterogeneous spectrum of different lymphoproliferative disorders may develop following organ transplantation, collectively referred to as post transplantation lymphoproliferative disorders (PTLD), ranging from reversible lesions to aggressive NHLs [Penn, 1990]. The overall risk for the development of NHL post-transplantation is approximately 25 to 50 times greater than expected, with the greatest risk in the first year posttransplantation, up to 120-fold (for cardiac transplantation) and 20 times higher (for renal transplantation), than the general population [Opelz and Henderson, 1993]. This period of highest risk, during the first year following transplantation, declines fairly rapidly in subsequent years and is significantly different from that noted in HIV, in which the risk increases over time. Further in contrast with the NHLs seen in the setting of HIV, these PTLDs are virtually uniformly associated with EBV and c-myc involvement is relatively rare.

The increased incidence of NHL in the context of organ transplantation has been noted both for solid organ transplantation as well as for bone marrow transplantation, for non-neoplastic hematologic diseases such as aplastic anemia. NHL has been observed more commonly in cardiac (5% incidence) than in renal transplant (2% incidence). The reason for this difference in incidence is not clear, but may be due to the more aggressive immunosuppressive therapy used in cardiac transplantation. Local immunologic factors may also affect the cell transformation process, since cardiac NHLs are more likely to develop following cardiac transplantation while kidney NHLs are more likely to develop after renal transplantation.

It has been noted that approximately 25–50% of all PTLDs may regress following withdrawal or decrease of immunosuppressive therapy [Starzl et al., 1984]. There is no clear way of predicting which would progress and which would regress, including morphologic and clonality assays. Recently, however, Knowles et al. [1995] have proposed a correlative morphologic and genetic analysis to define three apparently distinct categories of PTLDs. These three categories are: (1) plasmacytic hyperplasia, (2) polymorphic B-cell hyperplasia and polymorphic B-cell lymphoma, and (3) immunoblastic lymphoma or multiple myeloma. The features which distinguish these three processes are summarized in Table II. Accordingly, there appears to be a clearer understanding of the multistep process involved in the development of PTLDs, with one of the initial steps being the development of immortalized B-cell clones due to immunosuppressive therapy induced reactivation of latent EBV infection, or primary EBV infection. Indeed, primary EBV infection, rather than reactivation of latent infection, may be more important in this scenario [Savoie et al., 1994]. Oligoclones and monoclones with a proliferative advantage emerge from these polyclonal proliferations, and carry alterations in protooncogenes or tumor suppressor genes (for example N-ras and p53, respectively) leading to the development of a fully transformed B-cell malignancy.

Predictive factors. Given the risk of the development of NHL in this patient population, attempts have been made to identify predictive factors. A few such predictive factors have been identified including the intensity of therapy and the level of EBV infection. Regarding the

TABLE II. Categories of Post Transplant Lymphoproliferative Disorders[1]

	Plasmacytic hyperplasia	Polymorphous B-cell hyperplasia/ lymphoma	Immunoblastic lymphoma/ multiple myeloma
Site	oropharynx/nodal	nodal/extranodal	disseminated
Clonality[2]	polyclonal	monoclonal	monoclonal
EBV	variable[3]	single	single
Oncogene/tumor suppressor gene involvement[4]	—	—	+

[1]See reference 17.
[2]Based upon IgH gene rearrangement on Southern blotting.
[3]May be multiple or single copies.
[4]N-ras, p53 or c-myc.

intensity of immunosuppressive therapy, it has been shown that the amount of anti-lymphocyte globulin, anti-thymocyte globulin, OKT3 monoclonal antibody therapy, cyclosporin, and azathioprine is associated with an increased risk for the development of NHL [Opelz and Henderson, 1993]. Based upon the large body of evidence linking most cases of PTLD with EBV, attempts have been made to predict the development of PTLD using various assays of EBV infection. Serologic evaluation does not appear to have significant predictive value. By contrast, a direct evaluation of the level of EBV infection appears to be useful in determining which patients may develop overt NHL. One group [Savoie et al., 1994] measured the EBV load of peripheral blood lymphocytes, using PCR and Southern blotting, as well as quantitative lymphocyte culture. The load of EBV infected PBLs was shown to correlate with the risk of developing NHL. Another study [Preiksaitis et al., 1992] tried to quantitate the amount of EBV shed from the oropharynx using a quantitative dot-blot as well as PCR. The amount of shed EBV was indeed shown to be predictive for the development of NHL. This group also found that patients who developed PTLD were more likely to shed a strain of EBV (EBV1/EBV type A) which is known to transform B cells more efficiently than another strain (EBV2/EBV type B).

Congenital Immunodeficiency

Patients with congenital immunodeficiency syndromes have a 100-fold increased risk for the development of cancer [Mueller and Pizzo, 1995]. Approximately one-half of these cancers are likely to be NHLs. Unlike the NHLs identified in patients with HIV and PTLD, the biologic features of some of these diseases are not

TABLE III. Congenital Immunodeficiency Syndromes

	X-linked lympho-proliferative disorder	Wiskott Aldrich syndrome	Ataxia telan-giectasia
Inheritance	XLR	XLR	AR
% developing NHL	~35	~14	~10
EBV-associated	+++	+++	+/-
Site	extranodal	extranodal	nodal
Lineage	B	B > T	T > B

well characterized. Nevertheless, the risk remains significant, as summarized in Table III. In addition to the application of IgH VDJ PCR, described earlier, for the detection of emerging B-cell clones in some of these syndromes, a possible specific marker exists in patients with ataxia telangiectasia, who are at increased risk for the development of T-cell neoplasms. Non-random chromosomal abnormalities in these individuals may precede the emergence of a monoclonal T-cell population, and the detection thereof may have applications for screening purposes [Sherrington et al., 1994].

Autoimmune Diseases

Many patients with autoimmune diseases are at risk for the development of NHL [Kinlen, 1992]. In particular, patients with Sjogren's syndrome are at a very high risk for the development of a characteristic type of NHL, marginal zone B-cell lymphoma. Additionally, large epidemiologic studies have identified rheumatoid arthritis patients being at an increased risk for the development of NHL [Tennis et al., 1993]. While most of the NHLs seen in autoimmune diseases are B-cell lineage, other groups

suffer from T-cell lymphomas, as evidenced by the increased risk for the development of T-cell lymphomas, so called enteropathy associated T-cell lymphomas, in patients with celiac disease.

Although immunosuppressant drugs used for the treatment of autoimmune diseases clearly contribute to the development of NHL, this is not the only explanation. For example, patients with rheumatoid arthritis treated with azathioprine or cyclophosphamide have a 10-fold increased risk for the development of NHL, while those who have never been treated have only a 2.5-fold increased risk [Kinlen, 1992]. This is also observed in patients with Sjogren's syndrome, in which those receiving similar therapy have a 100-fold increased risk, while those not treated still have a very significant 36-fold increased risk. Given the major risk of development of NHL in this latter group, recent attempts have been made using a sensitive, seminested IgH PCR assay, in a small group of patients, to screen benign appearing lymphoid aggregates. Some of these lesions were indeed noted to be PCR positive in the absence of any other (morphologic, immunophenotypic, or Southern blot) evidence of neoplasia [Kinlen, 1992]. However, unfortunately, no follow-up was reported upon to determine whether these patients subsequently developed overt NHL.

OCCUPATIONAL/ENVIRONMENTAL FACTORS

There have been a variety of studies attempting to associate occupations and environmental factors with the development of NHL [Scherr et al., 1992]. While a variety of occupations and exposure to a number of environmental agents have been identified, perhaps the most compelling and exciting evidence has been found in farmers in rural Minnesota, who are exposed to herbicides and pesticides, such as phosphine, malathion, dichlorophenoxyacetic acid, chloropicrin, and captan. The incidence of NHL is increased approximately 5 to 10 times in these agricultural workers. Although the mechanism(s) involved in this remain unexplained, it appears that such workers have an "acquired genomic instability syndrome," analogous to the germline form of genomic instability found in patients with ataxia telangiectasia [Lipkowitz et al., 1992]. Specifically, this population is more likely (10- to 20-fold increase as compared with normal controls) to harbor hybrid antigen receptor genes (Tγ to Tβ) rearrangements (generated

as a consequence of the cytogenetic abnormality inv[7][p13q35]) in their peripheral blood lymphocytes. While these rearrangements are "innocent," without any defined transforming activity, this PCR based assay, analogous to that used for the detection of clonal IgH gene rearrangements, may provide a marker for VDJ recombinase activity, which is believed to be involved in the tumor specific, and transforming, cytogenetic abnormalities found in patients with NHL.

INFECTIONS

A number of different infectious agents may be involved in the pathogenesis of NHL, including EBV, HHV6, and HHV8, which appear to be primarily involved in the context of immunosuppression. However, there may also be specific infectious agents, HTLVI and Helicobacter pylori, which may be more directly involved in the development of subtypes of NHL.

HTLVI infection is associated with the development of adult T-cell leukemia/lymphoma, with a risk of 1% by age 40, and 4% by age 80, for the development of this neoplasm [Kondo et al., 1989]. However, although this retrovirus is an important inducing agent for NHL in certain geographic areas (particularly regions of the Caribbean and Japan), it does not appear to account for a significant fraction of NHLs in the United States. Recent evidence points to the bacterium Helicobacter pylori in the development a form of gastric NHL, MALToma [Parsonnet et al., 1994]. Functionally, in in vitro cell cultures, this organism has been known to have transforming ability. Furthermore, treatment with antibiotics has apparently resulted in complete regression of such neoplastic processes. Recently, these MALTomas have been shown to harbor non-random, specific molecular abnormalities, in particular trisomy of chromosome 3 [Wotherspoon et al., 1995]. The detection of trisomy 3 at the early stages of development of this tumor, via gastric washings, is potentially amenable to screening analysis using fluorescent in situ hybridization (FISH).

DISCUSSION

A variety of fairly well-defined groups at high risk for the development of a common event, NHL, the most common form of hematologic malignancy, have been clearly identified. The unravelling of the molecular genetic mechanisms that underly these malignancies has led

TABLE IV. Summary of Risk Groups and Potential Screening Markers

Risk group	Type of NHL	Marker
HIV+	High grade B-cell	IgH VDJ PCR t(8;14) PCR *ras* mutations CD4 counts IL-6 levels
PTLD	Spectrum of B-cell lesions	IgH VDJ PCR *ras* mutations *p53* mutations EBV PCR
Ataxia telangiectasia	Mostly T-cell	TCR V(D)J PCR inv(7) PCR t(14;14) PCR
Other congenital immunodeficiency syndromes	Mostly B-cell	IgH VDJ PCR
Autoimmune diseases	Mostly B-cell	IgH VDJ PCR
Agricultural workers	B-cell	inv(7) PCR
HTLV-I+	Adult T-cell leukemia/lymphoma	TCR V(D)J PCR
Helicobacter pylori	Gastric MALTomas	IgH VDJ PCR FISH of trisomy 3

to the development of extremely sensitive, and sometimes specific, tools that ought to have utility in screening these high risk groups, providing a scenario for possible chemotherapeutic intervention (Table IV).

However, unlike chemoprevention studies in solid tumors, early intervention in hematologic malignancies is still a developing field, and there is a dearth of appropriate clinical studies. Based upon the identification of the events and factors (such as EBV and IL-6) in the multistep pathway of neoplastic transformation, *in vitro* studies may provide clues to agents that may have a potential clinical role. Although EBV likely plays a significant role in the development of PTLDs and some HIV-associated NHLs, the efficacy of antiviral drugs such as acyclovir and ganciclovir in the therapy or prophylaxis is not established, since such therapy, while diminishing oropharyngeal shedding, has little effect on the EBV infected B cell burden in the peripheral blood. Rather, agents such as 5-azacytidine [Robertson et al., 1995] (to alter viral gene expression) and iridoids [Kapadia et al., 1996] may be useful chemopreventive agents. Also,

the effects of IL-6 in HIV-associated NHL may be abrogated, as has been shown in multiple myeloma, using murine monoclonal antibodies [Bataille et al., 1995] or suramin [Shiao et al., 1996]. These agents, together with the identification of the aforementioned defined groups at high risk for the development of these common neoplasms, would justify consideration of clinical chemoprevention trials, which, based upon the various molecular markers, should be relatively simple to study.

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