The mutator pathway is a feature of immunodeficiency-related lymphomas

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The mutator phenotype caused by defects in the mismatch repair system is observed in a subset of solid neoplasms characterized by widespread microsatellite instability-high (MSI-H). It is known to be very rare in non-Hodgkin lymphomas (NHL), whereas mutator NHL is the most frequent tumor subtype in mismatch repair-deficient mice. By screening a series of 603 human NHL with specific markers of the mutator phenotype, we found here 12 MSI-H cases (12/603, 2%). Of interest, we demonstrated that this phenotype was specifically associated with immunodeficiency-related lymphomas (ID-RL), because it was observed in both posttransplant lymphoproliferative disorders (9/111, 8.1%) and HIV infection-related lymphomas (3/128, 2.3%) but not in a large series of NHL arising in the general population (0/364) ($P < 0.0001$). The MSI pathway is known to lead to the production of hundreds of abnormal protein neoantigens that are generated in MSI-H neoplasms by frameshift mutations of a number of genes containing coding microsatellite sequences. As expected, MSI-H ID-RL were found to harbor such genetic alterations in 12 target genes with a putative role in lymphomagenesis. The observation that the MSI-H phenotype was restricted to HIV infection-related lymphomas and posttransplant lymphoproliferative disorders suggests the existence of the highly immunogenic mutator pathway as a novel oncogenic process in lymphomagenesis whose role is favored when host immunosurveillance is reduced. Because MSI-H-positive cases were found to be either Epstein–Barr virus-positive or -negative, the mutator pathway should act synergistically or not with this other oncogenic factor, playing an important role in ID-RL.

microsatellite instability

Cancers with a mutator phenotype constitute a frequent subset of solid tumors characterized by mismatch repair deficiency (1–4). These tumors exhibit a widespread genetic instability at the molecular level that mainly affects microsatellite sequences and are called MSI-H (microsatellite instability-high) tumors. Until now, this phenotype has been clearly demonstrated to occur in the hereditary nonpolyposis colorectal cancer syndrome, as well as in ≈10% of sporadic colorectal, gastric, and endometrial carcinomas (5–7). Clinically, microsatellite instability has been shown to be an independent factor of favorable outcome associated with a significant survival advantage in both gastric and colorectal cancers (8, 9). Because mismatch repair genes *Mlh1* or *Msh2* knock-out mice often develop lymphoid neoplasms (10), a number of groups looked for the presence of a mutator phenotype in different subgroups of human hematological malignancies including human non-Hodgkin lymphomas (NHL) (11–16). These studies have shown contradictory results concerning the prevalence of MSI in various histological categories and clinical entities of NHL due to the use of different criteria to define this tumor type. Thus, even if MSI was already reported sporadically in some cases of immunodeficiency-related lymphomas (12–14), its specific occurrence in such a particular clinical context has not been demonstrated until now.

Recently, stringent international criteria have been proposed to detect MSI-H tumors by using five microsatellite loci (17). Two of these five markers, Bat-25 and -26, were shown to be sufficient to determine the MSI-H phenotype with great accuracy and without the requirement for matching normal DNA (18, 19). Both these markers were used in the present study for the systematic screening of a large series of human NHL occurring in the general population and in acquired immunodeficiency patients related to HIV infection or iatrogenic immunosuppression after allograft. This approach allowed us to make some striking clinical correlations concerning the emergence of the mutator phenotype in NHL, because this phenotype was found to occur significantly only in immunodeficiency-related lymphomas (ID-RL), developed in either iatrogenically immunosuppressed or HIV-positive patients.

Materials and Methods

Patients and Specimens. Six hundred three cases of NHL from a retrospective collection of samples have been studied, including 518 frozen tissues biopsies and 85 peripheral blood samples. Ten institutions were involved in this study. The degree of lymphomatous infiltration of the biopsies was evaluated on a hematoxylin/eosin-stained frozen section and represented 60–80% of the tissue. All of the peripheral blood samples tested had a positive molecular detection of B cell clonality.

Abbreviations: MSI, microsatellite instability; MSI-H, MSI high; NHL, non-Hodgkin lymphoma; ID-RL, immunodeficiency-related lymphoma; PTLD, posttransplant lymphoproliferative disorder; EBV, Epstein–Barr virus; DLBCL, diffuse large B cell lymphoma; BL, Burkitt lymphoma.

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Table 1. Representation of the different NHL histological subtypes according to the international World Health Organization classification

Categories of lymphomas	Number of cases	Nonovert immunodeficient patients	Immunodeficient patients including (MSI-H cases)	
			HIV-related	Posttransplant recipients
B cell lymphomas				
LCL/CLL	85	85	0	0
FL	44	44	0	0
MCL	25	25	0	0
MZL	12	11	1	0
MZL/MALT	12	12	0	0
DLBCL	248	117	60 (2)	72 (5)
BL	93	43	46 (1)	4
PCNSL	13	0	8	5 (1)
P-PTLD	19	0	0	19 (1)
P-PTLD like	6	0	6	0
HL-like	2	0	0	2
Total (B-NHL)	559	337	121 (3)	102 (7)
T cell lymphomas				
PTCL unspecified	31	23	1	7 (2)
AILD	4	4	0	0
ALCL	3	0	3	0
Total (T-NHL)	38	27	4	7 (2)
Unknown	6	0	3	2
Total number of cases	603	364	128 (3)	111 (9)

LCL/CLL, lymphocytic lymphoma/chronic lymphocytic leukemia; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; MZL/MALT, MZL mucosis-associated lymphoid tissue; PCNSL, primary CNS lymphoma; P-PTLD, polymorphic PTLD; PTCL, peripheral T cell lymphoma; AILD, angioimmunoblastic lymphadenopathy; ALCL, anaplastic large cell lymphoma; PTCL, peripheral T cell lymphoma; B-NHL, B cell NHL; T-NHL, T cell NHL.

The series comprised 364 cases of NHL from the general population and 239 cases of ID-RL including 128 HIV infection-related lymphomas (HIV-RL) and 111 posttransplant lymphoproliferative disorders (PTLD). All of the cases were reviewed by at least one of the hematopathologists involved in the study, and lymphomas were classified according to the World Health Organization classification (Table 1) (20). Epstein–Barr virus (EBV) detection was performed in 214 of the 239 ID-RL cases [111 HIV-RL and 103 PTLD) and in 163 of the 364 NHL cases derived from the general population (25 chronic lymphocytic leukemia, 33 follicular lymphoma, 18 mantle cell lymphoma, 9 marginal zone lymphoma/mucosis-associated lymphoid tissue, 11 Burkitt lymphoma (BL), 52 diffuse large B cell lymphomas (DLBCL), and 15 T cell NHL] by *in situ* hybridization using Epstein–Barr-encoded RNA probes (Dakopatt Diagnostic, Trappes, France) as described (21) and immunoperoxidase detection of the latent membrane protein using the monoclonal antibody CS1a (Dakopatt Diagnostic) on paraffin sections from tumoral biopsies. A PCR technique using EBNA1 primers P1 (5′-CCTgTAggggAAgCCgAT-3′) and P2 (5′-CAATggTAAgACgACATT-3′) was performed as well for chronic lymphocytic leukemia samples.

Assessment for MSI. Extracted DNA from all tumoral and peripheral blood samples was screened for MSI by use of a PCR fluorescent-based genotyping method (ABI PRISM 3100 GA, Applied Biosystems) with both Bat-25 (NED labeled) and Bat-26 (FAM labeled) microsatellite markers (primer sequences are available on request). MSI-H tumors were defined as those with deletions of both these markers that have been shown to establish MSI status with an accuracy of >99.5% without the requirement of matching normal DNA, as described (18, 19). Samples with no instability at these markers were scored as microsatellite stable (MSS). Because rare polymorphisms of both these microsatellites have been described (19, 22, 23), specimens exhibiting instability at only one of these two mononucleotide markers were also classified as MSS.

Mutation Analysis at Target Genes for MSI. A total of 12 genes containing coding repeat sequences that have been already determined to be altered at variable frequencies in MSI-H cancers derived from different primary sites were analyzed for mutations in mutator NHL cases. They were selected for by a candidate gene approach among a number of other already described target genes for instability (24) because of their putative oncogenic role in lymphomagenesis (*TGFβRII*, *BAX*, *CASPASE-5, IGFIIR*, *TCF-4*, *BCL-10*, *β2-MICROGLOBULIN*, *BLM*, *RAD-50*, *GRB-14*, *MSH-3*, and *MSH-6*). PCR was performed with primers specific for each selected target gene (sequences available on request). The PCR products were separated on a 7 M urea/32% formamide/7% polyacrylamide gel, transferred overnight onto a Hybond *N* + nylon membrane, and hybridized with the ^{32}P-labeled antisense primer, in each case, as a probe.

Results

The Mutator Phenotype Is Rare in Human NHL. Twelve of 603 NHL cases (2%) exhibited deletions in both Bat-25 and -26 markers and were thus classified as MSI-H (Fig. 1). In six other cases, only one of these repeats was deleted, probably due to a rare polymorphism of these markers as described (19, 22, 23). Some normal allelic profiles in non-MSI NHL cases are shown in Fig. 2. These results confirm that MSI is a rare event in lymphomagenesis, as recently described (13, 16), but are in

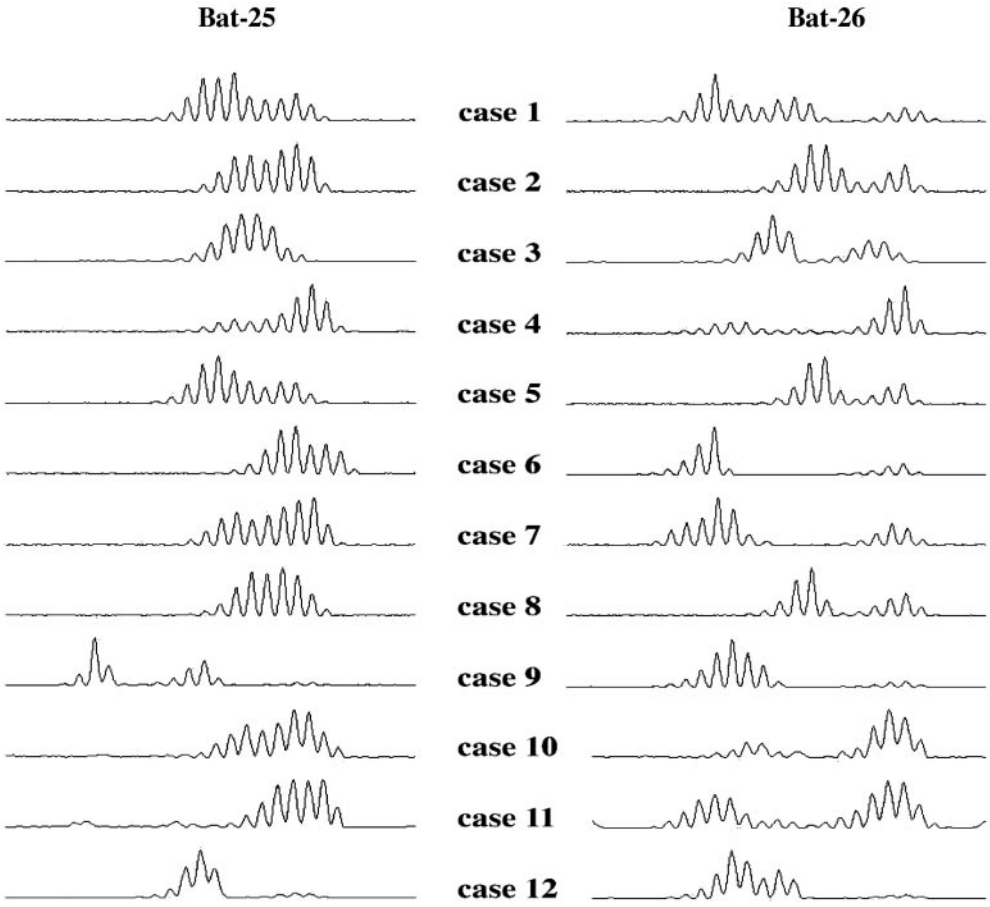

Fig. 1. Allelic profiles of Bat-26 and -25 obtained by multiplex fluorescent PCR of both these markers are shown for the 12 MSI-H NHL cases. In all cases, both normal alleles (from contaminating normal DNA) and shorter alleles (from tumor DNA) are present.

contrast with other studies in which the prevalence of such a tumoral phenotype has been overestimated in small series of human NHL by the use of nonconsensual microsatellite markers (11, 12, 15).

Microsatellite Instability Is a Feature of Immunodeficiency-Related Lymphomas. All MSI-H-positive cases were found in either HIV-infected ($n = 3/128$; 2.3%) or iatrogenically immunosuppressed patients after transplantation ($n = 9/111$; 8.1%). Because all MSI-H samples were ID-RL (12 of 239, 5%) compared to 0/364 cases of non-MSI NHL in immunocompetent patients ($P < 0.0001$; Fisher's exact test), this observation shows that the mutator pathway is a feature of immunodeficiency in human lymphomas. Because ID-RL are mostly represented by DLBCL and BL, we compared the occurrence of MSI in this latter group of aggressive lymphomas when developed in immunosuppressed patients or in the general population (e.g., 132 DLBCL and 50 BL vs. 117 DLBCL and 43 BL; Table 1). Here again, MSI association with host immunodeficiency was still significant ($P = 0.02$; Fisher's exact test). MSI-H ID-RL were found to be either of B or T cell origin. They included seven DLBCL, two peripheral T cell lymphoma, one polymorphic PTLD, one primary CNS lymphoma, and one BL.

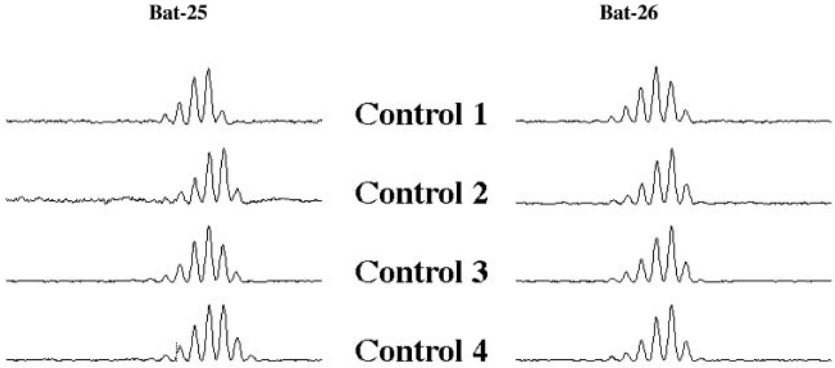

Fig. 2. Examples of monomorphic allelic profiles of Bat-26 and -25 in four non-MSI NHL are shown.

Table 2. Target gene frameshift mutations observed in MSI-H immunodeficiency-related NHL

	PTLD-1	PTLD-2	PTLD-3	PTLD-4	PTLD-5	HIV-NHL-1	HIV-NHL-2	HIV-NHL-3	Total number of alterations
RAD-50	−1	na	0	0	−1	−1	+1	+1	5/7
BAX	0	na	0	−1	0	+1	+1	0	3/7
CASPASE-5	0	na	−1	0	−1	+1	0	0	3/7
BLM	0	0	−1	0	0	−1	−1	0	3/8
MSH-6	0	0	−2	0	0	0	+1/+2	0	2/8
GRB-14	0	0	0	0	−1	0	0	−1	2/8
TGFBRII	0	na	+1	0	0	0	0	0	1/7
TCF-4	0	0	−1	0	0	0	0	0	1/8
IGFIIR	+2	0	0	0	0	0	0	0	1/8
MSH-3	0	0	0	0	0	0	−1	0	1/8
β2M	0	0	0	0	0	0	0	0	0/8
BCL-10	0	0	0	0	0	0	0	0	0/8

Base-pair insertions or deletions at coding repeats are indicated by numbers. na, nonamplified.

EBV Detection in MSI-H Immunodeficiency-Related Lymphomas. In lymphomas arising in the general population, the frequency of the association of NHL with EBV was low, as expected, without any significant detection in chronic lymphocytic leukemia, follicular lymphoma, mantle cell lymphoma, and marginal zone lymphoma/mucosis-associated lymphoid tissue. In BL, DLBCL, and T cell lymphomas [angioimmunoblastic lymphadenopathy and peripheral T cell lymphoma (PTCL)], positive cases for EBV were 17%, 27%, and 40%, respectively. On the contrary, ID-RL were associated with EBV with a higher frequency as described (21, 25): 52% in HIV infection-related lymphomas and 70% in PTLD. The EBV infection status was determined in 11 of the 12 MSI-H ID-RL, five cases (three DLBCL, one primary CNS lymphoma, and one polymorphic PTLD) were EBV positive, whereas six others (three DLBCL, three PTCL, and one BL) were EBV negative (Table 3).

Target Gene Frameshift Mutations in MSI-H Immunodeficiency-Related Lymphomas. *RAD-50, BAX, TGFβRII*, *IGFIIR*, *TCF-4*, *CASPASE-5*, *BLM*, *MSH6*, *MSH3*, *β2-MICROGLOBULINE*, *BCL-10*, and *GRB-14* (24) coding repeat sequences were analyzed for mutations in eight MSI-H ID-RL. Ten of these 12 target genes were altered at various frequencies in this series (Table 2). Seven of the eight MSI-H tumor samples tested showed at least one target gene mutation. We observed rare alterations in target genes such as *TGFβRII* or *TCF-4* that are frequently altered in MSI-H colorectal cancers. On the other hand, proapoptotic factors such as *BAX* or *CASPASE-5* showed relatively high mutational frequencies. Strikingly, the *RAD-50* gene that plays a role in repairing DNA double-strand breaks by homologous recombination and nonhomologous end joining (26) was frequently mutated in this series of MSI-H lymphomas (five of seven; 71.4%). According to these results, it seems that the target gene mutation profile associated with MSI-H ID-RL is specific for this tumor subtype, when compared to other MSI-H cancers such as gastrointestinal and endometrial tumors.

Discussion

Immunodeficiency, whether congenital, iatrogenic, or due to infection, increases the risk of a few types of cancers. The overall increment of NHL prevalence in acquired immunodeficiency after HIV infection or iatrogenic immunosuppression transplantation has highlighted this fact. Until now, such a phenomenon has been mainly explained by the etiopathogenic role of some oncogenic viruses such as EBV or human herpes virus type 8 that have been found associated with PTLD or with primary effusion lymphoma, initially described in HIV-infected patients, respectively (25, 27).

PTLD is one of the complications of solid organ transplantation that occurs in 2–5% of patients. Although PTLD may occur at any time, the majority of cases arise within the first 2 posttransplant years. It comprises a spectrum of lymphoproliferations ranging from early EBV-driven polyclonal proliferations to lymphomas of predominantly B or, less often, T cell type. Several categories have been identified according to their clinical presentation, morphology, and molecular features (28, 29). Most PTLD are associated with EBV, but EBV-negative cases also have been described and tend to occur later than EBV-positive ones (30). HIV-related NHL are mainly high-grade B cell-related neoplasms. Several pathogenic mechanisms and different histogeneses have been associated with these tumors according to the recent World Health Organization classification (20). Among this range of lymphomas, two main aspects are described: BL and DLBCL, having the features of centroblastic lymphoma or immunoblastic lymphomas (31). The oncogenic role of HIV seems to be indirect in such malignancies that have been associated with other oncogenic viruses such as EBV or Kaposi sarcoma herpes virus/human herpes virus type 8 (27). Genetic abnormalities also play a major role in such tumors involving activation of c-*MYC*, inactivation of *P53*, changes of *BCL-6* and, as described more recently, aberrant somatic hypermutations of protooncogenes already involved in the pathogenesis of lymphoïd malignancies (*PIM*-1, *PAX*-5, *RhoH*/*TTF*, and c-*MYC*) (32).

The dual properties of genetic instability and clonal expansion allow tumor development to occur in a microevolutionary fashion. A broad range of pressures, including host-related immunological factors, are exerted on a tumor during neoplastic development. Such pressures are responsible for the selection of genetic events providing growth or survival advantages to the tumor. As a consequence of microsatellite instability, tumor initiation and progression result from the accumulation of somatic mutations at repetitive microsatellite sequences contained in numerous target genes with a putative role in human cancers (24). Until now, no specific relationship has been established between this oncogenic pathway and host immunodeficiency. However, gastrointestinal MSI-H tumors are known to be inflammatory neoplasms with a high content of infiltrating lymphocytes (33). This clinical fact has been related to the presence of numerous neoantigens at the tumor cell surface as a consequence of the mutator process affecting numerous target genes and leading to the synthesis of truncated proteins during tumoral progression (34). In this context,

Table 3. Clinical data associated with MSI-H immunodeficiency-related NHL

Categories of lymphomas	Sex	Age*	Organ transplanted	IS regimen	Interval graft/PTLD, months	Localization	Phenotype and clonality	EBV	Survival period, months
PTLD									
DLBCL	M	39	Kidney	Cyclosporine + Imurel	84	Lymph node	B/M	+	30 (D)
DLBCL	F	26	Kidney	Cyclosporine + Imurel + corticoids	132	Abdominal mass	B/M	−	1 (D)
DLBCL	M	27	Kidney	Cyclosporine + Imurel	120	Lymph node	B/M	+	12 (A)
DLBCL	M	60	Heart	Cyclosporine + Imurel	60	Lymph node	B/M	+	8 (D)
DLBCL	M	3	Liver	Prograf	29	Stomach and jejunum	B/M	−	41 (A)
PCNSL	F	50	Lung	Prograf + Cellcept + Corticoids	75	Brain	B/M	+	1 (D)
P-PTLD	M	46	Kidney	Cyclosporine + Imurel + Corticoids	66	Lymph node and digestive tract	B/M	+	34 (D)
T-PTLD	M	69	Kidney	Imurel + Corticoids	360	Digestive tract and brain	T/M	−	
T-PTLD	M	70	Heart	Cyclosporine + Imurel + Corticoids	107	Digestive tract	T/M	−	14 (D)
HIV-related			CD4	High active antiretroviral therapy					
DLBCL	F	39	Low	None		Lymph node	B/M	−	
DLBCL	F	27	Low	None		Mediastinal mass	B/M	nd	
BL	M	36	Low	None		Lymph node	B/M	−	

M, male; F, female; D, dead; A, alive; P-PTLD, polymorphic PTLD; T-PTLD, T cell PTLD.
*Age (in years) at diagnosis of PTLD.

the fact that we demonstrate here a specific emergence of the mutator pathway in a subset of NHL arising in immunodeficient patients could be explained by a decrease in the capacity of the host to recognize and eliminate such highly immunogenic MSI-H lymphoid clones. This could be related to the fact that sporadic MSI-H gastrointestinal cancers are also known to be of later onset than non-MSI colorectal tumors, when there appears to be an age-related immunodeficiency. Because MSI-H-positive NHL cases were found here to be either EBV positive or negative (Table 3), the mutator pathway should act synergistically or not with this other oncogenic factor playing an important role during ID-RL tumoral progression.

Because one proposed mechanism for clonal evasion in MSI-H cancer cells was related to the alteration of the target *β2-MICROGLOBULIN* gene involved in antigen processing and presentation (35), we screened for *β2-MICROGLOBULIN* coding repeat mutations in MSI-H lymphomas but failed to find any in our tumor series (Table 2). On the contrary, our data suggest that the frequent alteration of the *RAD-50* gene, encoding a protein also involved in DNA repair, similar to genes involved in mismatch repair, might play an important role during MSI-H lymphoma progression, perhaps by favoring the overall instability process that characterizes these cancers. Inhibition of apoptosis by frameshift-inactivating mutations of proapoptotic factors such as *BAX* and *CASPASE-5* could also play an important role in these tumors, because we found mutations within these genes.

These data suggest the existence of a close clinical relationship between the emergence of the tumoral mutator phenotype and host immunodeficiency in a particular subset of tumors represented by human lymphomas. More generally, they also indicate that, as some oncogenic infectious factors, a cardinal feature of cancer, such as genetic instability, can be highly influenced by host immunity. This fact is of wide interest toward understanding the complex link between immunity and cancer.

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