Interleukin 2 Gene Polymorphisms Are Associated with Non-Hodgkin Lymphoma

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Non-Hodgkin lymphoma (NHL) is the most common hematologic malignancy worldwide. Interleukin-2 (IL-2) plays a key role in the proliferation of T cells and natural killer cells. It has been reported that polymorphisms in the *IL-2* gene are associated with various cancers. The aim of this study was to examine the effect of polymorphisms in the *IL-2* gene on the development of NHL in the Chinese population. *IL-2*-330T/G and +114T/G polymorphisms were detected by polymerase chain reaction–restriction fragment length polymorphism in 438 NHL cases and 482 age-matched healthy controls. Data were analyzed using the Chi-square test. Results showed that individuals with –330TG genotype or –330GG genotype had significantly increased susceptibility to NHL (Odds ratio [OR]=1.40, 95% confidence interval [CI]: 1.05–1.85, $p=0.020$ and OR=2.04, 95%CI: 1.28–3.24, $p=0.002$). Meanwhile, the +114T/G polymorphism did not show any correlation with NHL. When analyzing the haplotypes of these two polymorphisms, the prevalence of –330G/+114T haplotype was significantly higher in NHL cases than in controls (OR=1.45, 95%CI: 1.12–1.88, $p=0.005$). These data indicate that *IL-2* gene polymorphisms may be new risk factors for NHL.

Introduction

NON-HODGKIN LYMPHOMA (NHL) is the most common hematologic malignancy worldwide and has shown a pronounced increase in incidence in the past 10 years (Muller *et al.*, 2005). It represents 4% of all cancers and is the fifth most commonly diagnosed cancer in the United States (Muller *et al.*, 2005). Its rates are over 10/100,000 in the United States, Australia, and Western Europe, while <5/100,000 in Southern and Eastern Asia (Boyle and Levin, 2008).

Interleukin 2 (IL-2) secreted by Th1 cells plays a central role in the activation of T cell-mediated immune responses. It can augment natural killer (NK) cell cytolytic activity (Lenardo, 1991; Sakaguchi *et al.*, 2008). Also, IL-2 contributes to the development of regulatory T cells and regulates the expansion and apoptosis among activated T cells (Lenardo, 1991; D'Souza and Lefrancois, 2003). As for its relation to cancer, IL-2 has been reported to play an essential role in antitumor immunity (Zhang *et al.*, 2010). Clinical studies have shown that the transfection of the *IL-2* gene into tumor cells can enhance both specific and nonspecific antitumor immune responses (Okamoto *et al.*, 2003; Terao *et al.*, 2005). Recent research has demonstrated that IL-2 can induce an autologous graft-versus-lymphoma effect through activation of NK cells and cytotoxic T cells (Katsanis *et al.*, 1991) and may be used as an immunotherapy for NHL (Poiré *et al.*, 2010).

Two polymorphisms, –330T/G and +114T/G, have been identified in the *IL-2* gene. The –330T/G polymorphism lies in the promoter region of the *IL-2* gene and is implicated in the increased susceptibility to a range of inflammatory diseases and cancers, including gastric atrophy from *Helicobacter pylori* infection, rheumatoid arthritis, gastric cancer, and myelogenous leukemia (Amirzargar *et al.*, 2005; Pawlik *et al.*, 2005; Togawa *et al.*, 2005; Shin *et al.*, 2008; Wu *et al.*, 2009). However, no studies to date have examined the association between *IL-2* polymorphisms and NHL. Clarification of the relationship between *IL-2* polymorphisms and NHL may indicate a role of *IL-2* function in the etiology of NHL and provide clues to guide the treatment of this disease. To clarify this relationship, we have analyzed *IL-2*-330T/G and +114T/G polymorphisms in 438 cases of NHL and 482 controls.

Materials and Methods

Study subjects

This study included 438 patients with confirmed NHL and 482 healthy controls. The diagnosis of lymphoma was confirmed by histopathology and, in most cases, by supplementary immunohistochemistry analyses. Cases were categorized according to the Revised European–American Lymphoma Classification (Harris *et al.*, 1994). An additional

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assessment was performed at a later date to classify the samples according to the World Health Organization Classification (WHO, 2001). All patients and controls were consecutively recruited from Changhai Hospital and Shanghai East Hospital between February 2006 and May 2011. Subjects participating in the study were genetically unrelated Han Chinese. Written informed consent was obtained from all the subjects, and the study was performed with the approval of the Ethics Committees of Changhai Hospital and Shanghai East Hospital.

DNA extraction and genotyping

Genomic DNA was extracted from 5 mL frozen whole blood using the DNA Extraction Kit (Fastagen, China) according to the manufacturer's protocol. The *IL-2-330T/G* and *+114T/G* genotypes were determined using a polymerase chain reaction (PCR)-restriction fragment length polymorphism assay and DNA sequencing analysis. The PCR primers were designed as previously described (Matesanz *et al.*, 2001). The PCR primers for the *-330T/G* and *+114T/G* polymorphisms were 5'-ATTCA CATGTTCA GTGTAGTTCT-3' (forward) and 5'-GTGATAGC TCTAATTCATGC-3' (reverse); and 5'-ATGTACAGGATGCA ACTCCT-3' (forward) and 5'-TGGTGAGTTTGGGATTCTTG-3' (reverse), respectively. The PCRs were performed in a total volume of 25 μ L containing 100 ng genomic DNA, 20 pM of each primer, 0.2 mM dNTPs, 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM (NH₄)₂SO₄, 2 mM MgSO₄, 0.1% Triton X-100, and 1 unit of Taq polymerase (New England BioLabs). The PCR cycle conditions consisted of an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C; 45 s at 61°C for *-330T/G* and 63°C for *+114T/G*; 45 s at 72°C; and a final elongation at 72°C for 8 min. The PCR products were digested for 3 h at 37°C with the appropriate restriction enzymes (New England BioLabs). The restriction enzymes for the *-330T/G* and *+114T/G* genotypes were *BfaI* and *MvaI*, respectively. The digested PCR products were resolved on 8% acrylamide gels and stained with ethidium bromide for visualization under UV light. To confirm the genotyping results of the *IL-2-330T/G* and *+114T/G* polymorphisms, 18% of PCR-amplified DNA samples were examined by DNA sequencing. Results between PCR and DNA sequencing analysis were 100% concordant.

Statistical analysis

Genotype and allele frequencies of *IL-2* polymorphisms were compared between NHL cases and controls using the Chi-square test and odds ratios (OR), and 95% confidence intervals (CIs) were calculated to assess the relative risk conferred by a particular allele and genotype. Demographic and clinical data between groups were compared by Chi-square test and Student's *t*-test. Haplotypes of the two SNPs were conducted and analyzed using the SHEsis software, from the Web site <http://analysis.bio-x.cn/> (Bio-X Inc., Shanghai, China). Statistical significance was assumed at $p < 0.05$. The SPSS statistical software package version 13.0 was used for all of the statistical analyses.

Results

The frequency distributions of select characteristics of the cases and controls are presented in Table 1. The mean ages of

TABLE 1. CHARACTERISTICS OF THE SUBJECTS

Characteristics	Patients (n=438)	Controls (n=482)	P- Value
Mean age $\pm$ SD	56.6 $\pm$ 8.1	55.5 $\pm$ 7.9	0.851
Age (years) no. (%)			
≤ 60	307 (70.1)	314 (65.1)	0.110
> 60	131 (29.9)	168 (34.9)	
Sex no. (%)			
Male	255 (58.2)	269 (55.8)	0.461
Female	183 (41.8)	213 (44.2)	
Tumor size			
≤ 5 cm	360 (82.2)		
> 5 cm	78 (17.8)		
Immunohistological subtype no. (%)			
B-cell subtype	268 (61.2)		
Diffuse large B-cell lymphoma	175 (40.0)		
Small lymphocytic B-cell lymphoma	65 (14.8)		
Follicular lymphoma	10 (2.3)		
Other B-cell lymphoma	18 (4.1)		
T-cell subtype	170 (38.8)		
Peripheral T-cell lymphoma	112 (25.6)		
Angioimmunoblastic T-cell lymphoma	21 (4.8)		
Extranodal T/NK cell lymphoma	9 (2.1)		
Other T-cell lymphoma	28 (6.3)		
Ann Arbor stage n. (%)			
Stage I, II	131 (29.9)		
Stage III, IV	307 (70.1)		
Bone marrow involvement n. (%)			
No	328 (74.9)		
Yes	110 (25.1)		
B symptoms no. (%)			
With	197 (45.0)		
Without	241 (55.0)		
Lymphoma origin no. (%)			
Lymph node	246 (56.2)		
Extranodal origin	192 (43.8)		
Types of treatment no. (%)			
Chemotherapy	395 (90.1)		
Chemotherapy and radiation	31 (7.1)		
Chemotherapy and anti-CD20 mAb	9 (2.1)		
Autologous stem cell transplantation	3 (0.7)		

NK, natural killer.

the patient group and the control group were 56 years and 55 years, respectively. No significant differences were observed between the NHL cases and controls in regard to age ($p = 0.110$) and gender ($p = 0.461$).

The genotype and allele frequencies of the *IL-2-330T/G* and *+114T/G* polymorphisms in NHL cases and controls are summarized in Table 2. The genotype distributions of these two polymorphisms among the controls were in agreement with the Hardy-Weinberg equilibrium ($p > 0.05$). The T versus G allele frequencies of *IL-2-330* polymorphism were 59.1% versus 40.9% among the cases and 66.0% versus

TABLE 2. GENOTYPE AND ALLELE FREQUENCIES OF THE INTERLEUKIN-2 POLYMORPHISMS AMONG NON-HODGKIN LYMPHOMA PATIENTS AND CONTROLS

Polymorphism	NHL Cases n=438 (%)	Controls n=482 (%)	OR (95%CI)	p- Value
-330 T/G				
Genotype				
TT	136 (31.1)	193 (40.0)	Referent	
TG	246 (56.2)	250 (51.9)	1.40 (1.05–1.85)	0.020^a
GG	56 (12.7)	39 (8.1)	2.04 (1.28–3.24)	0.002^a
Allele				
T	518 (59.1)	636 (66.0)	Referent	
G	358 (40.9)	328 (34.0)	1.34 (1.11–1.62)	0.002^a
+114 T/G				
Genotype				
TT	128 (29.2)	138 (28.6)	Referent	
TG	220 (50.3)	240 (49.8)	0.99 (0.73–1.34)	0.939
GG	90 (20.5)	104 (21.6)	0.93 (0.64–1.35)	0.714
Allele				
T	476 (54.3)	516 (53.5)	Referent	
G	400 (45.7)	448 (46.5)	0.97 (0.81–1.16)	0.727
Haplotypes				
-330/+114				
TT	277 (31.6)	345 (35.8)	Referent	
TG	241 (27.5)	291 (30.2)	1.03 (0.82–1.30)	0.794
GT	199 (22.7)	171 (17.7)	1.45 (1.12–1.88)	0.005^a
GG	159 (18.2)	157 (16.3)	1.26 (0.96–1.66)	0.093

^ap-Values in bold are statistically significant ($p < 0.05$).

NHL, Non-Hodgkin lymphoma; OR, odds ratio; CI, confidence interval.

34.0% among the controls ($p = 0.002$). Prevalence of the *IL-2*-330TG and GG genotype were significantly higher in patients than in controls (OR=1.40, 95% CI: 1.05–1.85, $p = 0.020$; and OR=2.04, 95%CI: 1.28–3.24, $p = 0.002$). As for the +114T/G polymorphism, the prevalence of T and G allele were 54.3% and 45.7% in patients and 53.5% and 46.5% in controls ($p = 0.727$). The *IL-2* +114TG and GG genotype did not show any significant difference between the patients and the controls (OR=0.99, 95%CI: 0.73–1.34, $p = 0.939$ and OR=0.93, 95%CI: 0.64–1.35, $p = 0.714$). When analyzing the haplotypes of these two polymorphisms, 4 haplotypes were observed (Table 2). The -330G/+114T haplotype had significantly higher numbers in NHL cases compared with healthy controls (OR=1.45, 95%CI: 1.12–1.88, $p = 0.005$). These data suggest that both *IL-2*-330T/G polymorphism and -330G/+114T haplotype are associated with an increased susceptibility to NHL in the Chinese population.

We further evaluated the association of *IL-2*-330T/G and +114T/G polymorphisms with different clinical parameters in the 438 NHL patients (Table 3). Neither -330T/G polymorphism nor +114T/G polymorphism demonstrated significantly different genotype and allele distributions in regard to Ann Arbor stage (I+II vs. III+IV; $p > 0.05$), tumor size (≤ 5 cm vs. > 5 cm; $p > 0.05$), cell subtype (B cell vs. T cell) ($p > 0.05$), or bone marrow involvement ($p > 0.05$). Similarly, when analyzing the correlation of the two-polymorphism haplotypes with these clinical-pathological factors, we did not find any statistically significant association ($p > 0.05$). These results indicate that the two polymorphisms are not correlated with these clinical-pathological factors in NHL patients.

Discussion

Lymphomas are comprised of a series of heterogeneous malignant diseases that originate from lymphoid tissue; there is a great deal of variability in clinical-biological behavior exhibited by lymphomas (Pennanen *et al.*, 2008). The prognosis of this tumor is diverse and is dependent upon the immunohistological subtype, clinical staging, programs of treatment, and prognostic markers utilized. Studies have shown that *IL-2* may serve as a prognostic indicator in certain types of solid carcinomas, including lymphoma (Cooper *et al.*, 2001a). In the current study, we investigated the role of the *IL-2*-330T/G and +114T/G polymorphisms in susceptibility to NHL in the Chinese population and found for the first time that the *IL-2*-330T/G polymorphism and the -330G/+114T haplotype are risk factors for this disease.

Human genetic polymorphisms can play critical roles in various diseases (Wang *et al.*, 2011; Liu *et al.*, 2011; Ma *et al.*, 2011). Due to the importance of *IL-2*, polymorphisms in this gene have been extensively researched and two polymorphisms have been identified; one located in the promoter region at nucleotide -330 (John *et al.*, 1998) and another in the first exon at position +114 (Matesanz *et al.*, 2000). According to the HapMap Project and Environmental Genome Project, the frequency of the *IL-2*-330G allele is about 0.34 in Asian populations, which is consistent with our findings (G=34%). The -330T/G polymorphism has been shown to alter the level of *IL-2* and is associated with diseases and cancers, including gastric atrophy from *Helicobacter pylori* infection, rheumatoid arthritis, gastric cancer, and myelogenous leukemia (Amirzargar *et al.*, 2005; Pawlik *et al.*, 2005; Togawa *et al.*, 2005; Shin *et al.*, 2008; Wu *et al.*, 2009). Our data have shown that this polymorphism also affects the development of NHL. The other polymorphism, +114T/G, lies in the first exon of the *IL-2* gene. It is silent and does not produce any amino acid change. Wei *et al.* (2010) have reported that the distribution of the *IL-2* +114G allele is 46.7 in the western Chinese population, while our study has shown that this G allele is 46.5 in eastern Chinese. These data suggest that the distribution of this polymorphism is very similar in different Chinese populations. Since study has shown that some SNPs may be correlated with bone marrow involvement in NHL (Muller, *et al.*, 2005), we compared the two *IL-2* polymorphisms in NHL patients with or without bone marrow involvement (Table 3). However, we did not find any association between *IL-2* polymorphisms and NHL bone marrow involvement (Table 3). In addition, the two major cell subtypes of NHL, B-cell subtype and T-cell subtype, may have very different clinical characteristics. We therefore performed the stratification analysis of the *IL-2* polymorphisms between B-cell subtype group and T-cell subtype group (Table 3). Data did not show any significant difference between these groups. These results indicate that the *IL-2* polymorphisms may affect the whole NHL group rather than a specific subtype of this disease.

The mechanism in which the *IL-2*-330T/G polymorphism affects NHL remains unclear. *IL-2* is involved in the differentiation of B cells into plasmocytes, which could translate in B lymphoma. More importantly, *IL-2* promotes NK cell expansion (Meropol *et al.*, 1998) and enhances intrinsic NK cell

TABLE 3. ASSOCIATION OF INTERLEUKIN-2 POLYMORPHISMS WITH DIFFERENT CLINICAL PARAMETERS IN NON-HODGKIN LYMPHOMA

Polymorphism	Tumor size (%) ≤ 5 cm / > 5 cm (360)/(78)		OR 95%CI	p- Value	Ann Arbor stage (%) I + II / III + IV (131)/(307)		OR 95%CI	p- Value
-330 T/G								
TT	116 (32.2)	20 (25.6)	Referent		38 (29.0)	98 (31.9)	Referent	
TG	199 (55.3)	47 (60.3)	0.73 (0.41–1.29)	0.279	77 (58.8)	169 (55.0)	1.18 (0.74–1.86)	0.493
GG	45 (12.5)	11 (14.1)	0.71 (0.31–1.59)	0.398	16 (12.2)	40 (13.1)	1.03 (0.52–2.06)	0.930
T	431 (59.9)	87 (55.8)	Referent		153 (58.4)	365 (59.4)	Referent	
G	289 (40.1)	69 (44.2)	0.85 (0.60–1.20)	0.346	109 (41.6)	249 (40.6)	1.04 (0.78–1.40)	0.772
+114 T/G								
TT	106 (29.4)	22 (28.2)	Referent		42 (32.1)	86 (28.0)	Referent	
TG	182 (50.6)	38 (48.7)	0.99 (0.56–1.77)	0.984	65 (49.6)	155 (50.5)	0.86 (0.54–1.37)	0.524
GG	72 (20.0)	18 (23.1)	0.83 (0.42–1.66)	0.597	24 (18.3)	66 (21.5)	0.74 (0.41–1.35)	0.331
T	394 (54.7)	82 (52.6)	Referent		149 (56.9)	327 (53.3)	Referent	
G	326 (45.3)	74 (47.4)	0.92 (0.65–1.30)	0.624	113 (43.1)	287 (46.7)	0.86 (0.65–1.16)	0.326
Haplotypes								
-330 / +114								
TT	236 (32.8)	41 (26.3)	Referent		84 (32.1)	193 (31.4)	Referent	
TG	195 (27.1)	46 (29.5)	0.74 (0.46–1.17)	0.193	69 (26.3)	172 (28.0)	0.92 (0.63–1.35)	0.673
GT	158 (21.9)	41 (26.3)	0.67 (0.42–1.08)	0.098	65 (24.8)	134 (21.8)	1.12 (0.75–1.65)	0.587
GG	131 (18.2)	28 (17.9)	0.81 (0.48–1.38)	0.439	44 (16.8)	115 (18.8)	0.88 (0.57–1.35)	0.558
Polymorphism	Subtype (%) B cell/T cell (268)/(170)		OR 95%CI	p- value	Bone marrow involvement (%) No/Yes (328)/(110)		OR 95%CI	p- value
-330 T/G								
TT	116 (32.2)	20 (25.6)	Referent		38 (29.0)	98 (31.9)	Referent	
TG	199 (55.3)	47 (60.3)	0.73 (0.41–1.29)	0.279	77 (58.8)	169 (55.0)	1.18 (0.74–1.86)	0.493
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cytotoxicity (Eisenbeis *et al.*, 2004). NK cells are large granular lymphocytes that comprise 10% to 15% of peripheral blood lymphocytes (Cooper *et al.*, 2001b) and mediate antibody-dependent cellular cytotoxicity (ADCC) via expression of an activating receptor for the Fc portion of IgG antibodies (Cooper *et al.*, 2001a). Since ADCC plays critical roles against NHL, IL-2, one of the modulators of ADCC, may affect NHL and is considered as a possible therapy for this disease. It is reported that the G allele of the -330 polymorphism is correlated with reduced IL-2 production *in vivo* (Hoffmann *et al.*, 2001; Matesanz *et al.*, 2004). It is possible that the decreased IL-2 level can downregulate the antitumor response through ADCC in NHL patients and, therefore, increase the susceptibility to this disease.

There are some limitations to this study. For example, our patient sample size was relatively small. Our data might be of limited value and therefore, an additional study of more cases is needed. Since our study focused on the Chinese

population, it would be important to conduct a similar study in other ethnic populations. In addition, the current research studied two polymorphisms. It would be interesting to identify more polymorphisms in the *IL-2* gene and study their correlations with NHL.

In conclusion, this study investigated the allele and genotype frequencies of *IL-2* gene polymorphisms and identified the relationship between *IL-2* polymorphisms and susceptibility to NHL. The -330T/G polymorphism and -330G/+114T haplotype can act as potential risk markers for NHL and may assist in the design of therapeutic programs for this disease.

Acknowledgments

This research was funded by the Project of Modern Chinese Medicine, Shanghai Committee of Science and Technology, China (Grant No. 09dz1972400).

Disclosure Statement

No competing financial interests exist.

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Received for publication December 31, 2011; received in revised form February 14, 2012; accepted February 15, 2012.