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Characterization of the t(14;18) *BCL2-IGH* Translocation in Farmers Occupationally Exposed to Pesticides

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ABSTRACT

Increasing incidence of non-Hodgkin's lymphoma have been associated repeatedly with farming occupation and particular attention focused on the role of pesticide exposure to potentially explain part of this trend. A genetic hallmark of non-Hodgkin's lymphoma is the presence of recurrent chromosomal translocations involving the immunoglobulin heavy chain gene. Of these, the t(14;18), which deregulates *BCL2* expression and inhibits apoptosis, is the most frequent in follicular lymphoma and has been detected consistently in peripheral blood lymphocytes of healthy individuals. As *BCL2-IGH* translocation represents an early step of the malignant process, we evaluated the occurrence and molecular characteristics of *BCL2-IGH* translocation in 56 individuals occupationally exposed to pesticides in open field farming They were selected from a representative cohort of farmers with a well-defined assessment of pesticide exposure taking into account potential confounding factors, smoking, sunlight, and age. Our results suggest that occupational exposure to pesticides would increase *BCL2-IGH* prevalence together with the frequency of *BCL2-IGH*-bearing cells especially during the high pesticide use period. Distribution of *BCL2* or *IGH* breakpoint positions seemed to be independent of pesticide exposure and was similar to those found in other healthy populations or lymphoma patients. Finally, these results provide additional evidence that *BCL2-IGH* translocation measurements could be a measure of acquired genetic instability in relation to genotoxic exposure in a gene directly relevant in term of lymphomagenesis.

INTRODUCTION

Over the last 2 decades, the incidence of non-Hodgkin's lymphoma (NHL) has increased rapidly in most Western countries but the reasons for this trend are poorly understood (1, 2). Part of this increase may be attributed to established risk factors related to functional immunological abnormalities (immunosuppressive medication after organ transplantation, some autoimmune diseases like Sjögren syndrome or Ataxia Telangiectasia) or specific infectious agents (HIV, hepatitis C virus, and EBV), but cannot be explained only by these factors (3). Lifestyle and occupational factors have been addressed in several epidemiological studies and particular attention focused on the increased risks of NHL repeatedly observed among farmers (4, 5). Specific pesticide exposures have been widely investigated, and associations were found between risk of NHL and exposure to phenoxyacetic acid herbicides (6–8), triazine herbicides (9), carbamates (10), or organophosphate insecticides (11). In addition, evidence of a genotoxic effect linked to occupational exposure to pesticides has been accumulated in farmers by measuring cytogenetic endpoints or DNA damage using COMET assay (12–14). Studies designed to firmly establish some pesticides as causal agents of lymphoma have to deal with (*a*) the complexity of pesticide exposure including the diversity of chemical structures (partly due to the wide variety of agricultural activities); and (*b*) the diversity of potential toxic effects (cytotoxicity, immunotoxicity, and genotoxicity), which could lead to this effect.

Follicular lymphoma, one of the most common type of NHL accounting for 25–30% of cases, is characterized at 85% by the reciprocal translocation t(14;18)(q32;q21), which juxtaposes the *BCL2* gene on chromosome 18q21 near the immunoglobulin heavy chain (*IGH*) locus at chromosome 14q32 (15). Therefore, the *BCL2* gene is subjected to the control of the *IGH*-Eμ enhancer leading to the overexpression of the antiapoptotic BCL2 protein and increasing cell survival (16). Because of the involvement of the *IGH* locus as well as the presence of *de novo* nucleotides in *BCL2-IGH* junctions, the translocation was usually assumed to result, at least in part, from illegitimate V(D)J recombination in early B cells. But evidence to date has been mostly circumstantial, and recent breakpoint analysis tends to show that additional mechanisms could be involved (17).

Since 1994, many studies reported the presence at low frequencies of *BCL2-IGH* rearrangements in peripheral blood lymphocytes of healthy individuals (18, 19). Depending on PCR sensitivity, it was observed that from 50 to 80% of healthy individuals beard *BCL2-IGH* rearrangements with frequencies varying over a range of 100-fold (usually between 1×10^{-5} and 1×10^{-7}). In view of the initiating role of the *BCL2-IGH* translocation, it was hypothesized that individuals with an increased risk of lymphoma may have increased frequencies of *BCL2-IGH* translocation (20, 21). We then suggested that environmental factors (among which is occupational exposure to pesticides) could influence the prevalence, the frequency, or the molecular characteristics of *BCL2-IGH* bearing cells. In a study published previously, we found that neither the frequency of *BCL2-IGH*-bearing cells nor molecular characteristics of breakpoint junctions varied significantly in a healthy reference group over a 4-month period (between winter and spring), corresponding for farmers to the low or high pesticide use period, respectively (22). We used the same approach to characterize *BCL2-IGH* rearrangements on a population of farmers occupationally exposed to pesticides, mostly involved in open field farming, and coming from a representative cohort of agricultural population from the Calvados area and having a well-defined pesticide exposure assessment.

MATERIALS AND METHODS

Study Population. Located in Normandy, the Calvados is a geographical area of 5500 km^2 (1% of French territory) with 648,000 inhabitants (1% of French population). Eighty percent of its surface is devoted to agricultural activities, mainly open field farming and cattle breading, 22% for wheat and barley, 9% for corn, and >430,000 bovine cattle. A random sample of 8% the 7,991 farms of this area was generated and the farm owner of each selected farm was visited. In case of refusal, a short anonymous questionnaire on age and sex of the farm owner and on the farm characteristics (including pesticide use) was completed. No significant difference was observed between participants and refusals. During the 1997–2000 period, 410 farms (74% of participation rate) were included, and 758 subjects, corresponding to all of the adults on each participating farm (farm owners, spouses, agricultural workers, and retired), were surveyed. Biological samples were obtained in >90% of these individuals (one urine and blood sample allowing a biological collection of

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serum, urine extracts, viable lymphocytes, RBCs, DNA, leukocytes, and slides for cytogenetic analysis). A face-to-face comprehensive standardized questionnaire was administrated to all of the enrolled subjects with information about socio-demographic characteristics, tobacco and alcohol use, dietary, medical histories, and lifetime occupational exposure. A questionnaire specifically dedicated to farm characteristics and agricultural activities was also performed on each farm. Farmers were asked for detailed information regarding agricultural practices and exposure, including personal use of specific pesticides, timing and duration on each farm, years of exposure, specific crops and average acres planted, livestock, use of protective equipment, personal handling of products, brand name of products, spraying equipment, date of last pesticide application before biological sampling, and so forth. For this project, ethical approval was obtained from the local ethical committee (Comité Consultatif Pour les Personnes SE Prêtant à la Recherche Biomédicale).

For the present investigation, a subsample was selected among this cohort with the following criteria, males, without smoking history, and usual pesticide applicators on open field crops (Fig. 1). These criteria provided us with better conditions for data interpretation controlling for potential confounding factors (tobacco use) on a population of farmers with well-defined exposure assessment and who might be the most exposed to pesticides in the studied area (females only used insecticides on cattle or herbicides on farmyards). Among the 144 individuals matching these criteria, 56 were analyzed in the present study. Pesticide use varies considerably according to season with periods of high pesticide application (approximately from April to June in our area) and periods of little or no use. These two periods were designated as high pesticide and low pesticide use periods, respectively. All of the farmers ($n = 24$) from whom blood samples had been obtained during the high pesticide use period were examined, whereas 32 farmers were selected among the remaining 120 (Fig. 1). No significant differences were observed between the two groups for age ($P = 0.8$), farm area ($P = 0.5$), or farm area devoted to crops ($P = 0.9$) (Table 1). To control for potential season-related confounding factors, we collected individual daily sunlight data for the 1997–2000 period. The cumulative sunlight value (Joule/cm^2) for the 3-week period before sampling was calculated and ranged from 5,663 to 48,573 Joule/cm^2.

Blood Samples and DNA Preparation. Nurses collected at farmer homes a heparinized blood sample for each individual (35–40 ml). Peripheral blood lymphocytes were separated within 4 h by Ficoll Hypaque gradient centrifugation and stored in liquid nitrogen in RPMI/SVF 20%/DMSO 10%. DNA was extracted from peripheral blood lymphocyte using QIAamp DNA Blood Midi kit (Qiagen) according to the instructions of the manufacturer. Concentration of DNA was currently measured by UV spectrophotometry with determination of the ratio 260:280 nm (always between 1.7 and 2.0). For some samples, DNA concentration was also checked using fluorimetry. Data gave similar results and spectrophotometry were chosen for reliability.

Nested-PCR and DNA Sequencing for *BCL2-IGH* Translocation Status. *BCL2-IGH* fusions were amplified by nested PCR with a multitube approach using specific primers for the major breakpoint region in combination with a *JH* consensus primer, as described previously (20, 22). The reaction conditions were an initial step at 94°C for 10 min (Applied Biosystems, Foster City, CA), followed by 40 cycles of 1 min at 94°C, 1 min at 60°C, 1 min at 72°C, with a final extension step at 72°C for 5 min. One μl of the first PCR product was reamplified under the same conditions described above for 20 additional cycles. PCR products were analyzed on 1% agarose gel electrophoresis and visualized under UV light. For all of the samples, DNA integrity was checked by amplification of the albumin gene. Negative and positive controls were added in each PCR reaction, with, respectively, no added template and a diluted sample of the RL cell line characterized by a t(14;18) translocation. Among the 56 farmers selected for analysis, 3 of them were unavailable due to an insufficient amount of peripheral blood lymphocyte DNA. Finally, 53 samples were evaluated for the presence of *BCL2-IGH* translocation. Fresh PCR products were directly purified on a GFX PCR column (Amersham Biosciences, Piscataway, NJ) and sequenced using the Big Dye Terminator cycling sequencing Kit v3.0 (Applied Biosystems, Foster City, CA) on an ABIPRISM 377. As a unique and specific junction segment characterized each rearrangement, we excluded cross-contamination between samples.

Quantification of Translocation Frequencies. Translocation frequencies were estimated by a multitube approach and Poisson assumptions as described previously (20). For each sample, 30 replicates of 250 ng (equivalent to 40,000 cells) were amplified corresponding to a theoretical detection limit of 8.48×10^{-7}. In our multitube approach, an individual was considered to be positive when at least one replicate gave a positive signal on agarose gel electrophoresis that was confirmed to be specific by DNA sequencing. To allow comparisons between farmers and the reference group published previously (22), we had to be at an identical detection limit for the two groups (*e.g.*, 8.48×10^{-7}). The calculation of prevalence was made with the detection limit used in the present study, conducting to a new prevalence of 50% among referents.

Statistical Analysis. Median was used for comparison of the characteristics of farmers included or not included in the present study. Mean frequency value was calculated for all of the individuals. In this case, half of the detection limit (4.23×10^{-7}) was assigned for individuals with no detectable *BCL2-IGH* translocation. To take into account modifications of prevalence among compared groups of individuals, a second value of frequency was calculated for *BCL2-IGH* positive individuals. Because the distribution of *BCL2-IGH* frequency was skewed, values were log transformed for our analysis. For categorical variables, χ^2 or Fisher exact (when appropriate) tests were used. For continuous variables, Student *t* test or Wilcoxon nonparametric test and linear regression analyzes were performed using STATA software (STATA corporation release 7.0).

RESULTS

Characteristics of the Study Population. Table 1 shows the characteristics of the cohort ($n = 758$) and of the sub-sample selected for biological investigations analyzed ($n = 56$) or not ($n = 88$) in the present study (Fig. 1). The 56 analyzed farmers were slightly older (median age, 46 *versus* 41 years old; $P = 0.02$) and had a lower time since last pesticide use ($P = 0.09$). Except for the spraying equipment with the under-representation of the trailer sprayer among analyzed farmers, agricultural practices and exposure parameters used in this study (farm area, farm area devoted to crops, number of commercial products, and crop diversity) did not differ between the two groups. (Table 1).

Characterization of *BCL2-IGH* Translocation in Peripheral Blood Lymphocytes from Farmers. Prevalence of *BCL2-IGH* translocation in farmers occupationally exposed to pesticides was 71% (38 of 53). On the basis of the multitube approach, a frequency of *BCL2-IGH*-bearing cells was assigned to each farmer. The mean frequency of *BCL2-IGH* positive cells was 4.5×10^{-6} [$< 8.4 \times 10^{-6} - 57.6 \times 10^{-6}$] for all of the farmers and 6.1×10^{-6} when only positive farmers were considered.

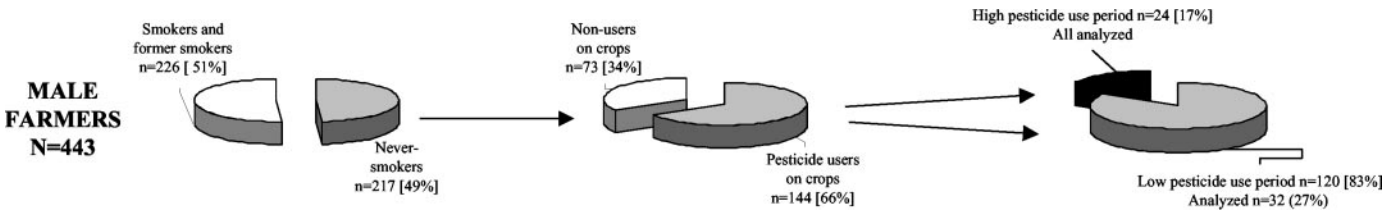

Fig. 1. Experimental design for the selection of farmers among the representative sample of agricultural population from the Calvados area. Following criteria of selection were retained: males, never-smokers, pesticide users on crops, and sampled either during the low or the high pesticide use period.

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Table 1 *Description of the characteristics of (A) the representative entire cohort of agricultural population from the Calvados area and of (B) selected male farmers analyzed or not in the present study*

	A Entire Cohort (n = 758)		B Selected male farmers (n = 144)	
	Females	Males	Analyzed farmers	Other identified farmers (not analyzed)
Characteristics	n = 315 (41.5%)	n = 443 (58.5%)	n = 56	n = 88
Median age [min–max]	46 [19–96]	44 [17–76]	46 [27–62]	41 [22–60]
Smoking habit				
Never-smokers	81%	49%	Never-smokers	Never-smokers
Current smokers	9%	26%		
Ex-smokers	10%	25%		
Medication				
No	53%	76%	71.4%	80.6%
Yes	47%	24%	28.6%	19.4%
Alcohol consumption				
None	21%	5.5%	2%	7%
Once a week	47%	37%	43%	40%
One meal/day	13%	16.5%	25%	18%
At all meals	17%	31%	21%	29.5%
More	2%	10%	9%	5.5%
Job title				
Farm owner	46%	78%	95%	92%
Agricultural worker	7%	10%	2%	7%
Retired	5%	7%	1.5%	1%
Spouse	38%	1.4%	1.5%	0%
Mean duration of agricultural employment (years ± SD)	27 ± 14 (1–59)	27 ± 12 (1–62)	28.5 ± 10 (5–48)	24.8 ± 10 (2–47)
Pesticide users on open field crops	0.6% (n = 2)	63%	100%	100%
Type of pesticide use				
Pesticides on open field crops	0.6%	63%	100%	100%
Herbicides on meadows	2%	52%	61%	74%
Insecticides on animals	55%	78%	85.5%	86.5%
Herbicides on farmyards	44%	66%	75%	78.5%
Herbicides on slopes	11%	66%	76.5%	76%
Mean duration of pesticide use (years ± SD)	13 ± 10 (6–20)	20 ± 9 (1–48)	20.6 ± 9 (1–41)	18.5 ± 9 (1–48)
Median farm area (hectares [min–max])[a]	97.5 (n = 2)	72.5 [0–406]	73.5 [12–213]	74.5 [0–290]
Median farm area devoted to crops[a]	78.5 (n = 2)	46 [0–403]	51 [12–208]	48 [0–203]
Median time since last pesticide exposure in days (min–max)	6 (n = 1)	28 (0–359)	28 (0–288)	44 (0–203)
Spraying equipment[a]				
None	0%	3%	0%	0%
Knapsack sprayer	50% (n = 1)	3%	0%	3.5%
Rear mounted sprayer	50% (n = 1)	54%	55.5%	62%
Trailer sprayer	0%	20%	37.5%	6%
Mixed or self propelling	0%	20%	9%	28.5%
Individual protective clothing[a,b]				
None	100%	47%	44.5%	41.5%
Mask only		9%	3.5%	13.5%
Gloves only		25%	31.5%	28%
Mask + gloves		19%	20.5%	17%
Crop diversity[a]				
Meadow + corn (1)		10%	4%	10%
Meadow or corn + wheat (2)		39%	35%	40%
Meadow or corn or wheat + peas (3)		7%	12.5%	6%
Meadow or corn or wheat or pea + sugar beet (4)		8.5%	12.5%	8%
Meadow or corn or wheat or pea or sugar beet + flax (5)		27%	29%	28%
Meadow or corn or wheat or pea or sugar beet or flax + truck farming (6)		8.5%	7%	9%

[a] Percentage shown only for pesticide users (n = 279, 277 men and 2 women).
[b] Individual protective device were used only during mixing loading tasks and not during application.

Seasonal Variation of *BCL2-IGH* Translocation. According to the day of blood collection, farmers were divided into two groups corresponding to the high or low pesticide use period. Consistence of this classification was confirmed by the median time since last pesticide use, which was significantly lower for individuals belonging to the high pesticide use period (3 days *versus* 55 days; $P = 0.0005$). As seen in Table 2, the prevalence of *BCL2-IGH* translocation was higher during the high pesticide use period, 81% (17 of 21) *versus* 65% (21

Table 2 *Characteristics of BCL2-IGH translocation in peripheral blood lymphocytes of farmers occupationally exposed to pesticides according to pesticide use period*

		Sampling period		
	Overall	Low pesticide use period (n = 32)	High pesticide use period (n = 21)	P[a]
Median time since last exposure (in days)	28	55	3	0.0005
BCL2-IGH prevalence[b]	71%	65%	81%	0.10
BCL2-IGH frequency (mean +/− SD)[c]	$4.5 \times 10^{-6} \pm 9.3 \times 10^{-6}$	$2.89 \times 10^{-6} \pm 4.1 \times 10^{-6}$	$6.94 \times 10^{-6} \pm 13.7 \times 10^{-6}$	0.12
BCL2-IGH frequency for positives farmers only[c] (mean +/− SD)	$6.1 \times 10^{-6} \pm 1.06 \times 10^{-5}$	$4.2 \times 10^{-6} \pm 4.6 \times 10^{-6}$	$8.5 \times 10^{-6} \pm 14.9 \times 10^{-6}$	0.10
Oligoclonality[d]	52.8% (17/36)	42.8% (9/21)	53.3% (8/15)	0.74

[a] P between the two sampling periods.
[b] Number of individuals with at least one positive specific PCR product.
[c] Results are expressed as the number of *BCL2-IGH* bearing cells in 10^6 normal cells measured with the Poisson's assumptions.
[d] Represents the number of individuals with multiple *BCL2-IGH* rearrangements on the number of analyzed positive individuals, as shown by DNA sequencing of junction sequences.

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of 32; $P = 0.10$). We also noted a nonsignificant ($P = 0.12$) increase of the mean frequency of *BCL2-IGH* translocation from 2.9×10^{-6} [$< 0.8 \times 10^{-6} - 15.7 \times 10^{-6}$] in the low pesticide use period to 6.9×10^{-6} [$< 0.8 \times 10^{-6} - 58 \times 10^{-6}$] in the high pesticide use period. When only positive individuals were included, the mean translocation frequency shared the same nonsignificant trend with period ($P = 0.10$). Despite a similar age distribution between the two groups [median age: 46 (range, 29–59) *versus* 47 (range: 27–62), for the low and the high pesticide use periods, respectively], a significant positive correlation was found with age only for the low pesticide use period ($P = 0.01$; $r^2 = 0.18$). Considering the whole group of farmers, a nonsignificant positive correlation was found with age ($P = 0.11$; $r^2 = 0.04$).

Because sunlight could contributed to the variations in *BCL2-IGH* frequency observed between the two periods, we addressed its influence by collecting daily sunlight data from the nearest meteorological station of each farm. The daily level of sunlight during the day of blood sampling is plotted in Fig. 2 for each analyzed farmer. As expected, the mean daily sunlight intensity exhibited a similar pattern than pesticide use and was higher during the high pesticide use period [1701 ± 663 J/m^2 (255–3105)] compared with the low pesticide use period [894 ± 668 J/m^2 (23–2882)]. However, no significant correlation was observed between *BCL2-IGH* frequency and sunlight radiation estimated with individual cumulative sunlight record the 1, 2, or 3 weeks before sampling ($P = 0.21$; $r^2 = 0.03$).

***BCL2-IGH* Translocation and Occupational Exposure Data.** To additionally investigate the relationship between *BCL2-IGH* frequency and pesticide exposure, we used several exposure parameters, including total farm area, farm area devoted to crops, and time since last exposure (as detailed in Table 1). Farmers with detectable *BCL2-IGH* translocation had a higher total farm area (97 *versus* 64 ha; $P = 0.01$), a higher farm area devoted to crops (65 *versus* 43 ha; $P = 0.03$), and a shorter mean time since last pesticide use (54 *versus* 89 days; $P = 0.08$). We then correlated exposure data with *BCL2-IGH* frequency. Significant positive correlations between frequency of *BCL2-IGH*-bearing cells and farm area ($P = 0.03$), and farm area devoted to crops ($P = 0.03$) were observed (Fig. 3). When analysis was performed separately for each pesticide use period, only farmers from the low pesticide use period demonstrated a significant positive correlation with farm area ($P = 0.006$; $r^2 = 0.23$) or farm area devoted to crops ($P = 0.04$; $r^2 = 0.13$). No correlation was observed between farm area and age ($P = 0.48$) excluding an influence of age for the observed effect. No significant relationship was found between *BCL2-IGH* translocation frequency and the number of commercial products used during the previous year, the type of spraying equipment, or the crop diversity.

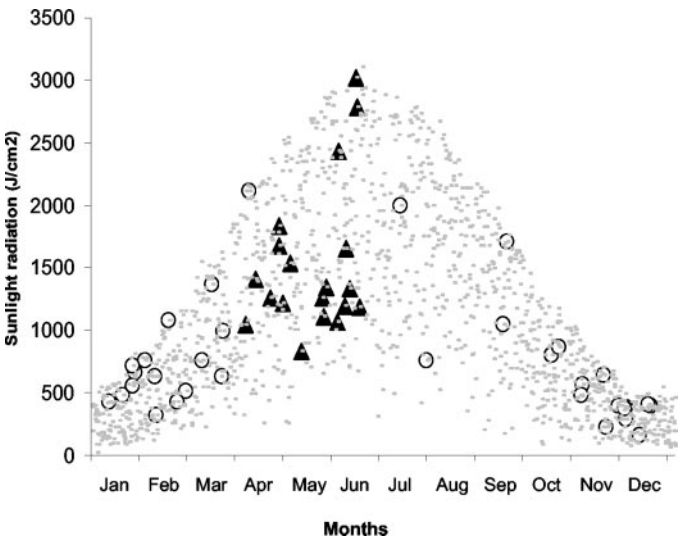

Fig. 2. Distribution of individual daily sunlight the day of blood sampling (expressed in Joule/cm^2) for analyzed farmers belonging to the low pesticide use period (○) or farmers belonging to the high pesticide use period (▲). All remaining daily sunlight records for the period 1997–2000 are indicated by *gray points.*

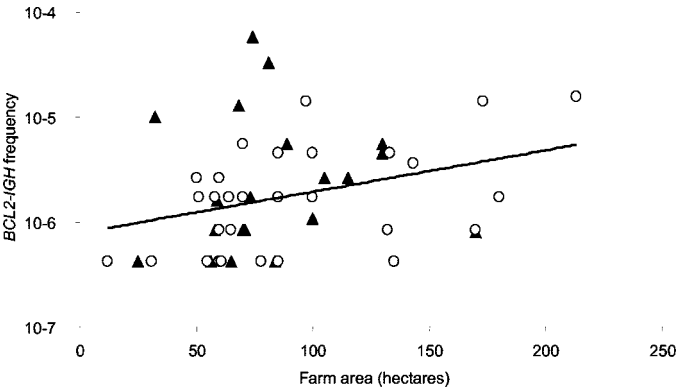

Fig. 3. Logarithm representation of *BCL2-IGH* frequencies with farm area in peripheral blood lymphocytes of farmers. (○), farmers sampled during the low pesticide use period; (▲), farmers sampled during the high pesticide use period; (—), trend curve for all farmers ($P = 0.03$; $r^2 = 0.09$)

There was no significant correlation between time since last pesticide use and frequency of *BCL2-IGH* translocations for the whole sample of farmers ($P = 0.12$; $r^2 = 0.05$). When the analysis was conducted separately for the two pesticide use periods, the results remained nonsignificant. To verify how the relationship between *BCL2-IGH* frequency and farm area for the low pesticide use period was influenced by the time since last pesticide use, we performed regression analysis for farmers having a time since last pesticide use either higher or lower to the median (*e.g.*, 50 days). The relationship remained significant inside the two subgroups, providing convincing evidence that the relationship between the frequency and the farm area was independent from the time since last pesticide use.

Molecular Characteristics of *BCL2-IGH* Translocation and Seasonal Variation. Sequence analysis performed in most positive individuals ($n = 36$ of 38) confirmed that amplified DNA fragments consisted of the juxtaposition of the *BCL2* oncogene near the *IGH* locus with an intervening sequence of nontemplated nucleotides. Of the farmers, 47.5% (17 of 36) presented with several *BCL2-IGH* rearrangements, the diversity of which was closely related to the frequency value. Consequently, oligoclonality was slightly higher during the high pesticide use period (Table 2). *BCL2*-major breakpoint region tended to fall into three clusters with an unbalanced use of the J6 segment. The frequency of breakpoints inside the three hotspot regions of the *BCL2* gene was similar for the low and the high pesticide use periods (82.4% *versus* 88.9%). Although there were slight discrepancies, such as an excess of breakpoint clustered in the region 3115 for farmers outside the pesticide use period (44% *versus* 25.9%) and a new microcluster at bp 3135 for the high pesticide use period, no major differences of breakpoint pattern were observed in the two populations (Fig. 4).

DISCUSSION

In the present study, we examined the occurrence and molecular characteristics of *BCL2-IGH* translocation in 56 individuals occupationally exposed to pesticides in open field farming taking into account potential confounding factors, smoking (23), sunlight (24), and age. Our results suggest that occupational exposure to pesticides

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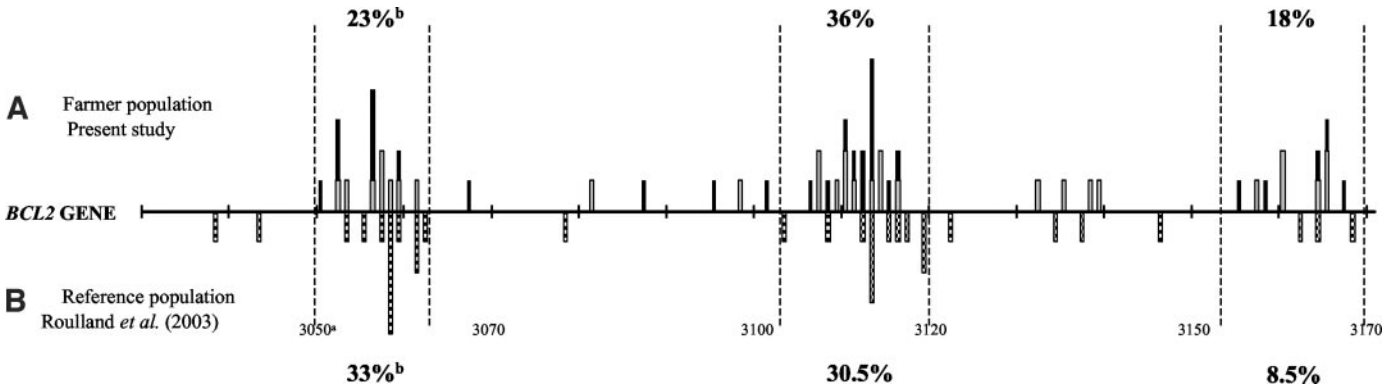

Fig. 4. *A*, distribution of *BCL2* breakpoints within the major breakpoint region in farmers according to the pesticide exposure period. Above the line, (▩) represents the high pesticide use period and (■) represents the low pesticide exposure period. *B*, distribution of *BCL2* breakpoints from the previously published reference population (⊡) is plotted to allow comparisons of *BCL2* breakpoints spectra (22). *a*, the positions of *BCL2* breakpoints are numbered according to the sequence M14745(39). *b*, percentage of breaks in each cluster is indicated *above the line* for the farmer group (23% outside the cluster region) and *below the line* for the reference group (28% outside the cluster regions).

would increase *BCL2-IGH* prevalence and the frequency of *BCL2-IGH*-bearing cells especially during the high pesticide use period.

Using a common sensitivity level of $\sim 1 \times 10^{-6}$ of translocation-bearing lymphocytes, studies conducted on healthy Caucasians without known exposure to genotoxins consistently reported that 50% of people present *BCL2-IGH*-bearing cells (19). When applying this level of sensitivity in our reference population (22), we observed a similar prevalence. In these conditions, farmers exhibited a 71% prevalence of *BCL2-IGH* translocation.

The mean frequency of *BCL2-IGH*-bearing cells was not significantly different among farmers compared with the reference population (~4 *BCL2-IGH*-positive cells in 1,000,000 normal cells). However, when we looked at dose-effect relationships between *BCL2-IGH* translocation and several pesticide exposure parameters, a positive correlation between the frequency and farm area was observed. This correlation was essentially due to the influence of the group of farmers sampled outside the high pesticide use period. Taken together, our results would indicate that a recent exposure induced a transient increase in prevalence and frequency of *BCL2-IGH* translocation during the high pesticide use period and mask a cumulative effect suggested by the relationship observed between farm area and frequency. The present data are highly consistent with previous results regarding the frequency of hybrid T-cell receptor γ/β antigen receptor-gene, mediated by the V(D)J recombinase complex, in peripheral blood lymphocytes from agricultural workers. A 10-fold increase of hybrid T-cell receptor γ/β frequency was observed in agricultural workers ($n = 12$) compared with control population ($n = 10$). In addition, the relative frequency of hybrid T-cell receptor γ/β was higher during their active pesticide exposure returning to baseline level after cessation of exposure (25). In the only study evaluating the relation between pesticide exposure and t(14;18) translocation in healthy farmers, no influence was found on t(14;18) prevalence that might be the result of a very low detected prevalence (8% for the whole population, 10% and 6% for the rural and the urban control group, respectively; 26).

The observed effect during the period of high pesticide use could be related to a direct induction of breaks on the *BCL2* gene. Genotoxic effects of at least some pesticides have been demonstrated by cytogenetic investigations conducted on farmers exposed to phosphine fumigators (27) or to a mixture of pesticides in open field farming (12) with an excess in breaks and rearrangements involving specific band 14q32 and 18q21 observed in comparison with control subjects (12). Epigenetic mechanisms might also be involved. Modification of chromatin structure by pesticide exposure could facilitate the accessibility to the *BCL2* and *IGH* loci, which are preferentially in close spatial proximity in normal B cells favoring the genesis of translocations (28). Apart from genotoxicity, some pesticides might also exhibit immunotoxic activities (29). *BCL2-IGH*-positive cells with an enhanced resistance to apoptosis due to BCL2 overexpression could be less sensitive to an immunosuppressive effect. Bell *et al.* (23) postulated that smoking induced the proliferation of pre-existing *BCL2-IGH* clones. This might be the same for pesticide exposure, as judged from the increased frequency and the similarity of clonal diversity, whatever the sampling period. Breakpoint distribution in farmers is similar to those published previously in follicular lymphoma (30, 31) and in other healthy individuals (22, 31, 32), which is in favor of a nongenotoxic effect. However, our PCR strategies targeted only the MBR region, which concerns 70% of the *BCL2* breakpoints in lymphoma. Other breakpoint clusters like *mcr* or *icr*, which seem to be more prevalent in follicular lymphoma than expected previously, were not considered (33).

We were concerned that the increased prevalence and frequency for farmers during the high pesticide use period could be influenced by other season-related environmental factors. Particularly, sunlight has been shown previously to influence the level of DNA damage or *BCL2-IGH* translocation frequency during summer (24, 34). In the present study, mean daily sunlight clearly displayed a similar yearly pattern than the pesticide exposure with a peak level of sunshine during the period of high pesticide use. However, analyzed farmers were sampled throughout the year, and no dose-effect relationships were found between *BCL2-IGH* frequency and individual daily or cumulative sunlight data, arguing against a role of sunlight in the observed frequency variations.

In conclusion, our results suggest for the first time that occupational exposure to pesticides might increase prevalence of positive individuals and frequency of *BCL2-IGH*-bearing cells, both as a consequence of cumulative exposure and transient effect of recent exposure. These results could be brought together with those of a recent case-control study on NHL showing that farming and exposure to selected pesticides (cyclodienes, lindane, captan, atrazine, and so forth) were strongly related to t(14;18)-positive NHL but not to t(14;18)-negative NHL (35). They support the interest of *BCL2-IGH* translocation in etiologic research. Studies focusing on chronic effect of pesticide exposure use different parameters to evaluate the intensity of pesticide exposure (farm area, duration of exposure, spraying equipment, and so forth), but none of these parameters have been firmly validated (36). Moreover, only recently has some integrative assessment of pesticide exposure been proposed (37). To confirm our results and to use a model that will be based on field exposure data (38), we will extend

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the study to the remaining 88 pesticide-exposed, never-smoking males from the cohort.

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Characterization of the t(14;18) *BCL2-IGH* Translocation in Farmers Occupationally Exposed to Pesticides

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