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Lung Cancer Risk: Effect of Dairy Farming and the Consequence of Removing that Occupational Exposure

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The aim of this study was to confirm the exposure-dependent reduction in lung cancer risk reported for dairy farmers exposed to endotoxin and to evaluate the consequence of leaving dairy farming and taking employment in industry or services, where exposure to microbial agents is lower. Standardized mortality ratios, with 95% confidence intervals, for 2,561 self-employed dairy farmers were estimated, considering the general population of Veneto, Italy, from 1970 to 1998 as the reference. Sixty-two lung cancer cases, whose information was checked against clinical records, were compared with 333 controls in a cohort-nested case-control study. Odds ratios with 95% confidence intervals were estimated by logistic regression analysis. A downward trend of standardized mortality ratios for lung cancer across tertiles of number of dairy cattle on the farm was significant ($p < 0.05$) from 1970 to 1984 but not from 1985 to 1998, when most subjects were no longer dairy farmers. Age- and smoking-adjusted odds ratios for lung cancer significantly decreased with increasing number of dairy cattle (p for trend $=$ 0.001) for workers for whom $\leq$15 but not $>$15 years had elapsed from the end of work to the end of follow-up. In conclusion, increased levels of endotoxin (or other associated environmental factors) might be protective against lung cancer; protection diminishes over time after that exposure is removed.

case-control studies; cohort studies; dairying; endotoxins; lung neoplasms; tumor necrosis factor-alpha

Abbreviations: CI, confidence interval; Th, T helper cell; TLR, toll-like receptor.

Studies have found that mortality from lung cancer in cotton textile industry workers decreased in proportion to increasing level (1) and increasing length (2, 3) of exposure to cotton dust. Enterline et al. (4) attributed this protective effect against lung cancer to endotoxin released by Gram-negative bacteria present in the cotton dust. The underlying mechanism may be activation of the immune system with macrophage surveillance and increased secretion of cytokines including tumor necrosis factor-alpha (5–7).

Previous studies (8–10) have shown that endotoxin and other microbial products are inherent elements of farm dust, particularly in cowsheds, where animal feces contaminate organic dusts on which bacteria and fungi adhere and grow. It is therefore necessary to divide "farmers" into "livestock/dairy farmers" and "crop/orchard farmers" because the former are exposed to much more endotoxin and related substances. Few studies compare the risk of lung cancer in these subcategories of farmers. In a case-control study (11), the odds ratio for lung cancer was 0.66 (95 percent confidence interval (CI): 0.48, 0.92) for dairy farmers, while a nonsignificant reduced risk (odds ratio $=$ 0.87, 95 percent CI: 0.64, 1.18) was found for crop/orchard farmers. In a cohort study (12), the standardized mortality ratio for lung cancer was 0.49 (95 percent CI: 0.31, 0.74) for dairy farmers compared with 0.81 (95 percent CI: 0.46, 1.31) for crop/orchard farmers. In a case-control study nested in the latter cohort (13), the age- and smoking-adjusted odds ratio for lung cancer decreased by 19 percent per head of dairy cattle

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(odds ratio = 0.81, 95 percent CI: 0.68, 0.97). Lastly, in a study based on US death certificates collected from 26 states from 1984 to 1993 (14), a rough adjustment for smoking resulted in lung cancer risks of 0.83 (95 percent CI: 0.79, 0.88) for livestock farmers and 0.95 (95 percent CI: 0.92, 0.97) for crop farmers. These studies suggest that differences in smoking habits do not entirely explain the reduced lung cancer risk for livestock/dairy farmers. Comparison of all of the above findings with those by Nieuwenhuijsen et al. (15), who reported personal exposure measurements in groups of California farmers (endotoxin levels averaging 132.5 endotoxin units per cubic meter (EU/m^3) of air during livestock farming compared with 19.9 EU/m^3 during field crop and fruit farming), suggests an inverse relation between lung cancer risk and endotoxin exposure.

In the Veneto region of Italy, the number of farmers has progressively declined over the last three decades. People leaving dairy farming and taking employment in industry or services have experienced a lower level of occupational exposure to bacteria and microbial agents. In this study, a cohort of dairy farmers was enrolled with the aim of investigating, in workers leaving dairy farming for other nonfarming employment, the health consequences, particularly regarding lung cancer mortality, of a change from a high to a low level of exposure to microbial agents.

MATERIALS AND METHODS

The source of this cohort is the historical archive of Italian self-employed small farmers, established in the 1950s when a new law (no. 1136/54) came into effect. The records include registry data (for the farm owner and his or her family members) and farm data (land area, type of crop, type and number of cattle housed), as well as any information on changes occurring over time. Farm owners are required by law to certify the date that their workers' employment ends (whether because of death, retirement, change of employment, or attendance at school) but not the date that farmwork commences. The entry date of farms from 1958 onward was considered as the entry date of the corresponding farmer into agricultural work. Other subjects were not included in the cohort unless some document was available certifying the particular subject as a farmer; the document date then became the entry date for the cohort. For farmers' sons, the date of commencement of agricultural work was set at their 14th birthdays unless there was any documented evidence of school attendance or other activities. In the archives, the records are divided according to the commune in which the farm is situated; therefore, we selected seven communes within the province of Vicenza, where dairy farming was the prevailing farm practice. All records of the seven communes were examined. According to the above criteria, we selected 2,916 male farmers for whom information was available on the dates of entry and exit from agricultural work and on the characteristics of the farms on which they worked.

Vital status was ascertained through the registry office of the communes of residence from January 1, 1970, to December 31, 1998. It was possible to trace 98.3 percent of the cohort, but 51 cohort members were lost to follow-up because they were unknown to the registry or had left the Veneto region. The number of study subjects was reduced to 2,692 by excluding those born before 1900 or commencing farmwork after 1994 and to 2,561 after excluding 131 crop/orchard farmers. For those who had died, cause of death was ascertained from the death certificates and was coded according to the *International Classification of Diseases*, Ninth Revision (16). Three deaths, the cause of which could not be ascertained, were considered "other causes unknown and unspecified" (*International Classification of Diseases*, Ninth Revision, code 799.9) and were included in the statistical analysis. According to the 2004 International Agency for Research on Cancer monograph on tobacco smoking (17), cancers of the oral cavity, pharynx (but not the nose, since there were no deaths in this study), larynx, esophagus, stomach, pancreas, liver, bladder and other urinary, and bone marrow (myeloid leukemia) were grouped together in a single nosologic category: all smoking-related cancer except lung.

Number of person-years was calculated by using the computer program PYRS (18), taking commencement of farmwork as the entry date and death or the end of follow-up as the exit date. The cause-specific mortality of the general population of the Veneto region was considered the reference, taking into account sex (males), age (stratified in 5-year groups), and calendar year (5-year periods: 1970–1974, 1975–1979, 1980–1984, 1985–1989, 1990–1994, 1995–1998). The standardized mortality ratio is the ratio between the observed and the expected numbers of deaths; the 95 percent confidence interval for the standardized mortality ratio was estimated on the basis of Poisson distribution.

Time-weighted averages of number of dairy cattle and land area of the farms were calculated if information on farm characteristics was updated after the first registration. The dairy farmers were then divided into three groups of a roughly equal number of subjects to determine tertiles of farm area by field (a local unit equal to about 0.4 hectare) or number of dairy cattle. The trend in standardized mortality ratios across the tertiles was analyzed by using the chi-square test for linear trend of the standardized mortality ratio (19).

To rule out a healthy-worker effect (a selection bias) and a confounding bias from smoking, a cohort-nested case-control study was carried out. A diagnosis of lung cancer recorded on 75 death certificates was checked against clinical records; 12 cases were excluded because their clinical records could not be traced, and one case was excluded because the lung tumor was secondary to a primary cancer elsewhere.

For control purposes, 333 dairy farmers (about four controls per case: 333/75) were selected by means of systematic sampling (one out of five) of cohort subjects known to be alive at the end of follow-up. The number of subjects in each tertile was 136 ((75 + 333)/3), and the odds of lung cancer in the first and third tertiles were set at 0.33 and 0.05, respectively; thus, the power was 99 percent to declare as statistically significant an odds ratio of 0.15 (0.05/0.33).

Calendar years of birth and of commencement and termination of farmwork, land area of the farm, and number of dairy cattle on the farm were obtained for both cases and controls from the files of self-employed small farmers in the province of Vicenza. Information on smoking (average number of cigarettes smoked lifelong for current smokers; average number of cigarettes smoked and number of years since quitting for former smokers) was assessed after 1998 through clinical records and phone interviews with the next of kin (wife, son or daughter) for cases. The same questions on smoking were addressed to controls through a mailed questionnaire. Nonresponders received a second and third letter, as required. Altogether, 283 dairy farmers answered (85 percent response rate). For two cases and 50 controls for whom smoking habits were unknown, occupational information was available.

By using the available quantitative measures of tobacco smoking, we coded smoking as 0 for nonsmokers; 1 and 2 for former smokers who had stopped smoking, respectively, more than and up to or including 10 years ago as of the date of death or end of follow-up; 3 and 4 for current smokers of, respectively, 1–10 and 11 or more cigarettes a day; 5 for current smokers of an unknown number of cigarettes; and 99 for those whose smoking habits were unknown. A parallel analysis was made by including and excluding the 52 subjects whose smoking habits were unknown.

Cases and controls were also categorized by tertiles of age, number of farm fields, and number of cattle on their respective farms. In a univariate analysis, the odds ratio for lung cancer, the 95 percent confidence interval, and the two-way *p* values were estimated by exact methods. When a variable was broken down into classes, the lowest class was considered the reference subgroup at a conventional risk of 1.0. The Cochrane-Armitage test for linear trend across the tertiles was performed by using the statistical package StatXact (20).

A dichotomous variable (1 for cases and 0 for controls) was the dependent variable, while number of fields, number of cattle (tertiles), and smoking habits (classified as above) were used as independent variables in a logistic regression analysis, stratifying by 5-year age groups. Stepwise multiple conditional logistic regression analysis (21) was used to select the variables to be included in the final model, where the odds ratio, exact 95 percent confidence interval, and exact error probability for a two-tailed test were estimated (conditional maximum likelihood estimate) by using the statistical package LogXact (22). Data on dairy farmers whose time intervals between ceasing work on farms and the end of follow-up (or death) were equal to or less or more than 15 years were analyzed separately.

A separate analysis was undertaken of two groups of dairy farmers older than 50 years of age: those who were current smokers or had given up smoking less than 10 years ago and whose time elapsed from the end of work to the end of follow-up (or death) was equal to or less or more than 15 years. The odds ratio (and 95 percent confidence interval) was estimated by conditional maximum likelihood estimate logistic regression (exact method) using the statistical package LogXact (22).

RESULTS

Table 1 shows that, for 2,561 dairy farmers, mortality from all causes was significantly lower than unity because of a lower standardized mortality ratio for infectious diseases, endocrine and metabolic diseases, tumors of all sites, diseases of the nervous and digestive systems, and accidents. The numbers of observed deaths from tumors of the esophagus, pancreas, lung, and bladder were significantly lower than those expected from regional rates.

Table 2 lists the number of active dairy farmers in any calendar year before 1970, at the beginning (1970) and end (1998) of follow-up, and at a time point intermediate between the two (1985). Cessation of dairy farming was strongly associated with a low number of cattle and/or a small farm size. After 1984, subjects in the first two tertiles were mainly former farmers.

In table 3, mortality data are broken down by tertiles of cattle housed and calendar periods, as well as results of the chi-square test for trend of the standardized mortality ratio across tertiles. A significant ($p < 0.05$) decrease with increasing number of cattle was observed for lung cancer (but not for all smoking-related cancer except lung) from 1970 to 1984, accidents and external causes from 1985 to 1998 (probably due to occupational changes by former dairy farmers), and circulatory disease in both observation periods. In table 3, total cancer mortality may be obtained by reviewing the values in the second and third rows and total mortality by checking the values in rows 2–6.

Similar results were obtained when mortality data were broken down by tertiles of farmland area and calendar periods (data not shown).

The results of the cohort-nested case-control study for lung cancer are shown in tables 4–9. Table 4 shows that, compared with controls, cases used more tobacco, were older, had a smaller farm area, and had fewer dairy cattle. Univariate odds ratios for lung cancer were significantly increased for current and former smokers and for older age groups but were significantly reduced with increasing farm area and number of cattle.

Table 5 shows the numbers of exposed cases and controls and the results of the logistic regression analysis of 235 dairy farmers who had ceased farmwork less than 15 years before the end of follow-up or death. The age- and smoking-adjusted odds ratio for lung cancer was significantly lower in the second and third tertiles relative to the first, and it significantly decreased with increasing number of dairy cattle on the farm (*p* for trend across tertiles = 0.0007). The variable ''farmland size'' did not have an independent influence in addition to that of ''dairy cattle on farm'' entered in the stepwise logistic regression model. These results remained the same when we included in the analysis the 24 subjects for whom data on smoking habits were missing (data not shown).

We used two logistic regression models. Table 6 reports the results of the first model; data were included on 22 squamous cell carcinoma cases and 190 controls (for all, $\leq$15 years had elapsed since the end of work). Table 7 reports the results of the second model in which we included information on 23 lung carcinoma cases of other and unknown histology and 190 controls (again, for all, $\leq$15 years had passed since the end of work). Results are similar in both tables, suggesting an exposure-dependent reduction of lung cancer risk across tertiles of dairy cattle.

The occupational variables (dairy cattle and farmland size) were not entered in the stepwise logistic regression

TABLE 1. Mortality in a cohort of 2,561 dairy farmers followed up between 1970 and 1998, Veneto region, Italy

Cause of death	Observed/ expected (no.)	SMR†	95% CI†
All causes	882/1,162.3	0.76*	0.71, 0.81
Tumors	246/369.8	0.67*	0.58, 0.75
Malignant tumors	244/362.3	0.67*	0.59, 0.76
Buccal pharynx	9/16.34	0.55	0.25, 1.05
Esophagus	4/13.79	0.29*	0.08, 0.74
Stomach	20/30.78	0.65	0.40, 1.00
Colon	12/20.16	0.60	0.31, 1.04
Rectum	16/11.21	1.43	0.82, 2.32
Liver biliary	22/22.70	0.97	0.61, 1.47
Pancreas	7/15.06	0.46*	0.19, 0.96
Other digestive	4/4.96	0.81	0.22, 2.06
Larynx	9/12.74	0.71	0.32, 1.34
Lung	75/116.4	0.64*	0.51, 0.81
Connective tissue	1/0.68	1.47	0.02, 8.18
Cutaneous melanoma	2/2.25	0.89	0.10, 3.21
Other skin	3/1.17	2.56	0.52, 7.49
Prostate	16/23.31	0.69	0.39, 1.15
Bladder	1/14.01	0.07*	0.00, 0.40
Other urinary	7/8.32	0.84	0.34, 1.73
Brain	2/4.83	0.41	0.05, 1.49
Lymphosarcoma	7/6.15	1.14	0.46, 2.35
Multiple myeloma	3/3.95	0.76	0.15, 2.22
Leukemia	9/9.22	0.98	0.45, 1.85
Other and unspecified	11/18.80	0.59	0.29, 1.05
Infective	3/8.81	0.34*	0.07, 0.99
Endocrine	10/23.32	0.43*	0.21, 0.79
Blood	5/2.60	1.92	0.62, 4.49
Mental	2/8.70	0.23*	0.03, 0.83
Nervous system	8/17.38	0.46*	0.20, 0.91
Circulatory	420/466.4	0.90*	0.81, 0.99
Respiratory	68/85.20	0.80	0.62, 1.01
Digestive	42/82.02	0.51*	0.36, 0.68
Genitourinary	7/14.35	0.49	0.20, 1.01
Musculoskeletal	1/1.72	0.58	0.01, 3.23
Ill-defined causes	23/11.17	2.06*	1.31, 3.09
Accidents	47/67.25	0.70*	0.51, 0.93

* $p < 0.05$.

† SMR, standardized mortality ratio; CI, confidence interval.

TABLE 2. Number of active dairy farmers during specified calendar years, by tertile of dairy cattle on the farm or farmland area, between 1950 and 1998, Veneto region, Italy

Calendar year	Tertile of dairy cattle on the farm			Total
	I	II	III	
Any before 1970	888	824	849	2,561
1970	571	541	477	1,589
1985	146	238	559	943
1998	35	85	421	541
	Tertile of farmland area			
	I	II	III	
Any before 1970	821	902	838	2,561
1970	525	571	493	1,589
1985	185	345	413	943
1998	57	200	284	541

model when analyzing the remaining 108 dairy farmers for whom more than 15 years had elapsed from the end of work to the end of follow-up or death (table 8).

Table 9 shows the results of the regression analysis of a group of 73 dairy farmers over 50 years of age who were current smokers or who had stopped smoking less than 10 years ago and had ceased farmwork less than 15 years before the end of follow-up or death. The age- and smoking-adjusted odds ratio for lung cancer significantly decreased with increasing number of dairy cattle (p for trend across the tertiles $= 0.0012$) and was significantly lower than 1.0 in the third relative to the first tertile.

DISCUSSION

Interpretation of a standardized mortality ratio of less than 1.00 is not straightforward, because this decrease can also be attributed to negative biases: either a healthy-worker effect (selection bias) or differences in smoking habits (confounding bias). With its greater physical demands, an occupation such as farming requires good health. A selection bias arises when farmers are compared with the general population, which includes unemployed disabled persons and/or those of poorer health. Although confounding may be controlled for during the analysis provided that the relevant information has been collected, selection bias cannot (23), and we therefore performed a cohort-nested case-control study.

On the basis of the principles of Checkoway et al. (24), we used the "cumulative incidence sampling" approach in our nested case-control study, which involves selecting controls from those persons free of the disease under consideration at the end of the study period. If the disease being studied is rare, this procedure produces an odds ratio estimate approximately equal to the risk ratio. The deviation from the risk ratio becomes substantial only when the cumulative incidence over the study period is greater than 10 percent. Because the cumulative incidence of lung cancer was about 2.4 percent (62/2,561) in dairy farmers, our odds ratio approximated the risk ratio.

Of 12 cases excluded from our study (because their clinical records could not be traced), none worked with 14 or more and eight worked with four or less dairy cows. If the latter had been included in the reference category shown

TABLE 3. Mortality data for 2,561 dairy farmers, by tertile of number of dairy cattle on the farm and calendar period (1970–1984 and 1985–1998), Veneto region, Italy

Nosologic category	Calendar period	1–4 dairy cattle			5–10 dairy cattle			≥11 dairy cattle			χ^2 trend†
		Observed/ expected (no.)	SMR	95% CI‡	Observed/ expected (no.)	SMR	95% CI	Observed/ expected (no.)	SMR	95% CI	
Smoking-related cancer, except lung§	1970–1984	17/28.96	0.59*	0.34, 0.94	18/21.59	0.83	0.49, 1.32	1/9.09	0.11*	0.00, 0.61	0.85
	1985–1998	18/31.38	0.57*	0.34, 0.91	18/29.71	0.61*	0.36, 0.96	11/17.49	0.63	0.31, 1.13	0.06
Lung cancer (*a*)	1970–1984	19/25.03	0.76	0.46, 1.19	7/18.71	0.37*	0.15, 0.77	2/7.79	0.26*	0.03, 0.93	3.96*
	1985–1998	22/25.38	0.87	0.55, 1.33	18/24.48	0.74	0.44, 1.16	7/15.00	0.47*	0.19, 0.96	2.11
Other-site cancer except lung (*b*)	1970–1984	31/49.93	0.62*	0.42, 0.88	30/37.45	0.80	0.54, 1.14	3/15.50	0.19*	0.04, 0.56	1.30
	1985–1998	42/58.16	0.72*	0.52, 0.98	41/54.77	0.75	0.54, 1.02	22/30.20	0.73	0.46, 1.10	0.00
Accidents (*c*)	1970–1984	12/15.41	0.78	0.40, 1.36	9/13.06	0.69	0.32, 1.31	6/9.20	0.65	0.24, 1.42	0.14
	1985–1998	14/10.57	1.32	0.72, 2.22	5/10.42	0.48	0.16, 1.12	1/8.61	0.12*	0.00, 0.65	10.59*
Circulatory diseases (*d*)	1970–1984	104/100.5	1.03	0.85, 1.25	74/74.83	0.99	0.78, 1.24	15/27.98	0.54*	0.30, 0.88	4.17*
	1985–1998	116/115.2	1.01	0.83, 1.21	79/104.6	0.76*	0.60, 0.94	32/44.37	0.72	0.49, 1.02	4.44*
All causes except ($a + b + c + d$)	1970–1984	50/57.46	0.87	0.65, 1.15	28/43.52	0.64*	0.43, 0.93	14/18.40	0.76	0.42, 1.28	0.72
	1985–1998	38/62.43	0.61*	0.43, 0.84	30/56.99	0.53*	0.36, 0.75	11/26.42	0.42*	0.21, 0.74	1.30

* $p < 0.05$.

† Chi-square test for trend of the standardized mortality ratio (SMR).

‡ CI, confidence interval.

§ Includes cancers of the oral cavity, pharynx, larynx, esophagus, stomach, pancreas, liver, bladder and other urinary, and bone marrow (myeloid leukemia).

in table 4, the odds ratio for lung cancer would have been even less than the 0.18 found for farmers working with 14 or more dairy cattle. Therefore, the bias is an underestimation.

For our cases and controls, information on smoking habits was missing for 52 and was available for 343 dairy farmers. However, the distribution by number of cattle was similar in both groups so that, when the former were excluded from the logistic regression model, the results were unchanged.

Lastly, the influence of age, a confounder in any study of lung cancer risk, was controlled for by conducting age-stratified logistic regression analyses.

Results of the cohort study suggest an exposure-dependent reduction in the risk of lung cancer for dairy farmers during the observation period from 1970 to 1984, when most subjects worked at their farms. Interestingly, this pattern was not found for all smoking-related cancer except lung or for all other-site cancer excluding lung (table 3). Results of the nested case-control study confirmed that a recent exposure to dairy farm dust protected against lung cancer (table 5) when we controlled for the influence of age and smoking habits. Such an exposure afforded protection from lung cancer, even for subjects older than 50 years of age who were current smokers or had ceased smoking less than 10 years ago (table 9, third tertile of number of dairy cattle).

Our findings agree with those reviewed earlier (11–14) and with those reported for other similarly exposed populations. From 1977 to 1985 in Iceland, the standardized mortality ratio for lung cancer was 0.53 (95 percent CI: 0.30, 0.87) for farmers who mainly raise sheep and/or cattle (25). Dairy products accounted for 57.1 percent of the commodities sold by New York State farmers in 1979 (26), and the incidence of lung cancer was 0.52 (95 percent CI: 0.42, 0.63) of that expected for male (27), and 0.33 (95 percent CI: 0.20, 0.51) for female (28), New York State farmers. Most investigators believe that smokers are at a higher risk of developing squamous cell lung carcinoma than adenocarcinoma. However, in a cohort of 254,417 Swedish farmers, the rate ratio of lung cancer was equally decreased for adenocarcinoma (rate ratio = 0.39, 95 percent CI: 0.33, 0.47) and squamous cell carcinoma (rate ratio = 0.35, 95 percent CI: 0.33, 0.38) (29). Dairy farming is predominant in Scandinavia (30), and farmers in the above cohort were mainly dairy farmers.

Lastly, experimental and clinical evidence shows that endotoxin (31–33) and mycobacterial components (34) inhibit tumor activity, particularly for very small tumors.

The basis of the inverse relation between exposure to endotoxin (and possibly to other microbial components) and lung cancer risk is unknown, but there are two possibilities, which are not mutually exclusive. The first is a direct pharmacologic effect of endotoxin because it is a potent stimulator of endogenous antineoplastic mediators (7). The major mechanism of such a direct action is macrophage activation with expression of tumor necrosis factor-alpha and other mediators. Tumor necrosis factor-alpha has a direct cytotoxic

TABLE 4. Results of the case-control study nested in a cohort of dairy farmers followed up between 1970 and 1998, Veneto region, Italy

Study variables	Cases (no.)	Controls (no.)	OR*	95% CI*	Trend-test *p* value†
Smoking habits					
Nonsmoker	1	138	1.00		
Former smoker (>10 years since cessation)	6	61	13.6	1.57, 628.7	
Former smoker (≤10 years since cessation)	13	20	89.7	11.8, 3,837	
Current smoker (1–10 cigarettes/day)	8	23	48.0	5.78, 2,144	
Current smoker (≥11 cigarettes/day)	25	33	104.5	15.6, 4,322	
Current smoker (no. of cigarettes unknown)	7	8	120.7	12.1, 5,472	
Unknown	2	50	5.52	0.28, 328.0	
Age at end of follow-up or death (years)					
<52	1	129	1.00		<0.001
52–67	22	111	25.6	3.97, 1,063	
≥68	39	93	54.1	8.73, 2,207	
No. of dairy cattle					
1–4	32	94	1.00		<0.001
5–13	22	108	0.60	0.31, 1.15	
≥14	8	131	0.18	0.07, 0.42	
No. of fields					
<11	33	85	1.00		<0.001
11–21	20	124	0.42	0.21, 0.81	
≥22	9	124	0.19	0.08, 0.43	

* OR, odds ratio; CI, exact 95% confidence interval.

† Exact value for the two-tailed Cochrane Armitage trend test.

action on tumor cells and activates tumoricidal macrophages, cytotoxic T cells, natural killer cells, and neutrophils (6).

The other possibility is that exposure to certain microbial populations primes the immune system for a particular pattern of responses. Evidence exists that normal maturation of the immune system requires stimulation of members of a class of evolutionarily conserved transmembrane proteins, termed toll-like receptors (TLRs), that are found in many eukaryotic forms of life. More than 10 TLRs are found on human cells, and two—TLR2 and TLR4—are activated by certain commonly occurring microbial components, or adjuvants, including endotoxin from Gram-negative bacteria, mycobacterial lipopeptides, and fungal glucans (35). In particular, these adjuvants cause dendritic cells to mature into well-regulated and highly effective antigen-presenting cells, thereby enabling these cells (which are part of the innate immune system) to play a central role in regulation of the adaptive immune responses to a wide range of antigens.

Another important effect of TLR activation is regulating the maturation of T helper cells (Th) into two main classes termed Th1 and Th2, which, by producing or inducing different cytokines, cause quite different immune responses. It has been shown that activation of TLRs by bacteria, including ubiquitous saprophytic mycobacteria, or by bacterial components, notably endotoxin of Gram-negative bacteria, enhances Th1 expression and inhibits Th2-mediated allergic reactions (36, 37). Some cancers have likewise been associated with a "Th2 drift," and, although the question of cause and effect remains, preliminary evidence shows that a therapeutically induced shift from Th2 to Th1 immune reactivity is associated with a more favorable prognosis in patients with melanoma (38). Intravesical instillation of Bacillus Calmette-Guérin is a widely used and effective treatment for superficial bladder cancer, and at least one mechanism is induction of the Th1 cytokine interleukin-12 by activation of TLR2 and TLR4 by Bacillus Calmette-Guérin (39). As shown in table 1, despite the relatively small number of cases, the reduction in the risk of bladder cancer in this study was particularly marked.

The pharmacologic and immunologic effects of endotoxin may be closely interdependent because the action of tumor necrosis factor-alpha (which, as mentioned above, is a key mediator of the antineoplastic action of endotoxin) on cells and tissues is critically dependent on the balance between Th1- and Th2-mediated immune reactivity (40). Accordingly, the pharmacologic action of endotoxin may require an underlying immune regulation determined by prior exposure to this and perhaps other microbial components. In our study, the persistence of a protective effect for up to 15 years after removal of a major occupational source of appropriate microbial agents suggests that some form of immunologic memory is involved.

From 1985 to 1998, when our subjects were mainly former dairy farmers (table 2), an exposure-dependent protective

TABLE 5. Numbers of exposed cases and controls among 235 subjects for whom ≤15 years had elapsed from the end of work to the end of follow-up (or death) in the case-control study nested in a cohort of dairy farmers followed up between 1970 and 1998, Veneto region, Italy†

Terms	Cases (no.)	Controls (no.)	OR	95% CI	*p* value
Smoking habits					
Nonsmoker	0	99	1.00		
Former smoker (>10 years since cessation)	2	37	3.37	0.25, +INF‡	0.355
Former smoker (≤10 years since cessation)	11	12	47.27	6.73, +INF	<0.001
Current smoker (1–10 cigarettes/day)	8	20	27.72	3.70, +INF	<0.001
Current smoker (≥11 cigarettes/day)	19	19	162.4	23.6, +INF	<0.001
Current smoker (no. of cigarettes unknown)	5	3	66.21	6.60, +INF	<0.001
No. of dairy cattle*					
≤4	24	31	1.00		
5–13	14	55	0.28	0.07, 0.94	0.039
≥14	7	104	0.10	0.02, 0.48	0.002

* $p = 0.0007$: trend for odds ratio across the tertiles of number of dairy cattle.

† Lung cancer risk in relation to smoking habits and number of dairy cattle, estimated by using conditional logistic regression analysis for stratified data (5-year age groups): model terms (Terms), odds ratio (OR), exact 95% confidence interval (CI), and exact error probability (*p*) for a two-tailed test.

‡ INF, infinity.

effect against lung cancer was not observed (table 3). Likewise, in the nested case-control study, with correction for the effects of age and smoking, there was no evidence for an exposure-dependent reduction in lung cancer risk for farmers who had ceased working on dairy farms more than 15 years previously (table 8). Thus, protection afforded by exposure to endotoxin-containing organic dusts diminishes over time after that exposure is removed.

When epidemiologic evidence on cancer risk among textile industry workers was reviewed in relation to calendar year of study publication (which may be considered a surrogate for the calendar years of exposure), lung cancer risk was approximately 0.4 in the first epidemiologic study on cotton workers (published by Kennaway and Kennaway in 1936), about 0.7 in subsequent studies (mostly published in the 1970s and 1980s), and approximately 1.0 in a later study

TABLE 6. Information on 22 squamous cell carcinoma cases and 190 controls for whom ≤15 years had elapsed from the end of work to the end of follow-up (or death) in the case-control study nested in a cohort of dairy farmers followed up between 1970 and 1998, Veneto region, Italy†

Terms	Cases (no.)	Controls (no.)	OR	95% CI	*p* value
Smoking habits					
Nonsmoker	0	99	1.00		
Former smoker (>10 years since cessation)	1	37	1.79	0.04, +INF‡	0.7162
Former smoker (≤10 years since cessation)	6	12	28.0	3.66, +INF	0.0006
Current smoker (1–10 cigarettes/day)	3	20	11.2	1.09, +INF	0.0419
Current smoker (≥11 cigarettes/day)	10	19	93.3	12.7, +INF	<0.00001
Current smoker (no. of cigarettes unknown)	2	3	38.5	2.67, +INF	0.0089
No. of dairy cattle*					
≤4	9	31	1.00		
5–13	8	55	0.27	0.04, 1.37	0.1369
≥14	5	104	0.11	0.02, 0.72	0.0152

* $p = 0.0093$: trend for odds ratio across the tertiles of number of dairy cattle.

† Lung cancer risk in relation to smoking habits and number of dairy cattle, estimated by using conditional logistic regression analysis for stratified data (5-year age groups): model terms (Terms), odds ratio (OR), exact 95% confidence interval (CI), and exact error probability (*p*) for a two-tailed test.

‡ INF, infinity.

TABLE 7. Information on 23 cases whose lung carcinoma was of other and unknown histology and 190 controls for whom ≤15 years had elapsed from the end of work to the end of follow-up (or death) in the case-control study nested in a cohort of dairy farmers followed up between 1970 and 1998, Veneto region, Italy†

Terms	Cases (no.)	Controls (no.)	OR	95% CI	*p* value
Smoking habits					
Nonsmoker	0	99	1.00		
Former smoker (>10 years since cessation)	1	37	0.96	0.02, +INF‡	1.000
Former smoker (≤10 years since cessation)	5	12	17.6	2.09, +INF	0.0065
Current smoker (1–10 cigarettes/day)	5	20	13.4	1.60, +INF	0.0145
Current smoker (≥11 cigarettes/day)	9	19	55.1	7.55, +INF	<0.00001
Current smoker (no. of cigarettes unknown)	3	3	17.6	1.54, + INF	0.0203
No. of dairy cattle*					
≤4	15	31	1.00		
5–13	6	55	0.24	0.04, 1.08	0.0660
≥14	2	104	0.08	0.00, 0.91	0.0371

* $p = 0.0051$: trend for odds ratio across the tertiles of number of dairy cattle.

† Lung cancer risk in relation to smoking habits and number of dairy cattle, estimated by using conditional logistic regression analysis for stratified data (5-year age groups): model terms (Terms), odds ratio (OR), exact 95% confidence interval (CI), and exact error probability (*p*) for a two-tailed test.

‡ INF, infinity.

(published by Wang et al. in 1995); when they are ordered chronologically, "inconsistent" cancer risks are no longer contradictory because they conform to the principle that the determinant of an effect is the concentration of a substance (41). The time-related differences could be due to a lowering of the dust concentration in the workplaces. Accordingly, measures introduced to prevent respiratory disease may, paradoxically, have increased the lung cancer burden among the textile workers.

Evidence is growing that various environmental changes over the last few decades have deprived the population, particularly in the industrially developed nations, of microbial challenges to the immune system that would otherwise have occurred regularly throughout life (42–44). According

TABLE 8. Information on 108 subjects for whom >15 years had elapsed from the end of work to the end of follow-up (or death) in the case-control study nested in a cohort of dairy farmers followed up between 1970 and 1998, Veneto region, Italy†

Terms	Cases (no.)	Controls (no.)	OR	95% CI	*p* value
Smoking habits					
Nonsmoker	1	39	1.00		
Former smoker (>10 years since cessation)	4	24	3.92	0.28, 234.0	0.499
Former smoker (≤10 years since cessation)	2	8	12.49	0.42, 1,091	0.192
Current smoker (1–10 cigarettes/day)	0	3	242.5	–INF,‡ 9,457	1.000
Current smoker (≥11 cigarettes/day)	6	14	30.8	2.31, 2,076	0.004
Current smoker (no. of cigarettes unknown)	2	5	24.63	0.78, 2,322	0.075
No. of dairy cattle*					
≤4	7	43	1.00		
5–13	8	35	2.67	0.54, 15.7	0.296
≥14	0	15	0.76	–INF, 7.23	0.833

* $p > 0.05$: trend for odds ratio across the tertiles of number of dairy cattle.

† Lung cancer risk in relation to smoking habits and number of dairy cattle, estimated by using conditional logistic regression analysis for stratified data (5-year age groups): model terms (Terms), odds ratio (OR), exact 95% confidence interval (CI), and exact error probability (*p*) for a two-tailed test.

‡ INF, infinity.

TABLE 9. Numbers of cases and controls among 73 subjects aged >50 years, current or former smokers, for whom ≤15 years had elapsed from the end of work to the end of follow-up (or death) in the case-control study nested in a cohort of dairy farmers followed up between 1970 and 1998, Veneto region, Italy†

Terms	Cases (no.)	Controls (no.)	OR	95% CI	*p* value
Smoking habits					
Former smoker (≤10 years since cessation)	11	11	1.00		
Current smoker (1–10 cigarettes/day)	8	11	0.48	0.08, 2.46	0.511
Current smoker (≥11 cigarettes/day)	19	6	3.69	0.75, 22.2	0.127
Current smoker (no. of cigarettes unknown)	5	2	2.61	0.17, 64.3	0.812
No. of dairy cattle*					
≤4	23	6	1.00		
5–13	14	12	0.28	0.06, 1.12	0.077
≥14	6	12	0.08	0.01, 0.48	0.003

* $p = 0.0012$: trend for odds ratio across the tertiles of number of dairy cattle.

† Lung cancer risk in relation to smoking habits and number of dairy cattle, estimated by using conditional logistic regression analysis for stratified data (5-year age groups): model terms (Terms), odds ratio (OR), exact 95% confidence interval (CI), and exact error probability (*p*) for a two-tailed test.

to this, the "hygiene hypothesis," such deprivation has resulted in a failure of immunoregulatory pathways and networks to develop normally. In this context, a number of environmental factors, such as living on farms and in large families and attending day-care facilities, protect against allergy (44) and also against leukemia (45, 46). In addition, certain vaccinations (against tuberculosis and smallpox) and infectious diseases protect against melanoma (47, 48).

Other studies suggest that endotoxin reduces the fat component of body mass (49) and arterial pressure (50); accordingly, we found an inverse relation between exposure to farm dust containing endotoxin-like substances and risk of circulatory disease (table 3).

In conclusion, on the basis of the mainly indirect evidence presented in this paper, we postulate that increased levels of endotoxin (or other environmental factors associated with endotoxin) protect against lung cancer; protection diminishes over time after that exposure is removed. A better understanding of the underlying immunologic mechanisms is needed.

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