

Association between clinical symptoms and lymphocyte abnormalities in a population with chronic domestic exposure to industrial solvent-contaminated domestic water supply and a high incidence of leukaemia

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Summary. An unusually high incidence of leukaemia and recurrent infections was noted in children exposed in utero to domestic water supply contaminated with industrial solvents including trichloroethylene, perchloroethylene and 1,2-*trans*-dichloroethylene. Medical and laboratory investigations were carried out on 28 family members of the patients with leukaemia with particular emphasis on the immunological system to determine if they displayed symptoms associated with acute or chronic exposure to these chlorinated hydrocarbons. The principal organ systems affected were neurological, immunological and cardiocirculatory. Damage to these systems was found in all subjects by history, physical and laboratory parameters. Damage to the immunological system was manifest by altered ratios of T lymphocyte subpopulations, increased incidence of auto-antibodies, increased infections and recurrent rashes.

Introduction

Between 1964 and 1967, two auxiliary water wells were opened to meet the additional domestic and industrial water requirements of the population of Woburn, Mass., a town of 37 000. They tapped an aquifer separate from that supplying the majority of the town, and over the next 15 years these wells (G and H), pumped intermittently according to the overall demand on the main municipal well-field. One area in East Woburn, with a population of 10 000 to 15 000, received most of the water from these wells [18]. In 1979 it was discovered that the soil, ground water and well water were contaminated with industrial solvents and the wells were closed. Trichloroethylene (TCE) was the main volatile organic found (267 ppb), with lesser amounts of tetrachloroethylene (21 ppb), and 1,2-*trans*-dichloroethylene, 1,1,1-trichloroethane and chloroform [5]. By hydrogeological modelling it has been estimated that the contamination was present several years prior to discovery. Water from the main well-field was not contaminated [18].

Between 1969 and 1979, 12 cases of leukaemia occurred in Woburn when only 5.3 were expected. This excess was due to 6 cases occurring in one of the town's six consensus tracts [10, 13]. The incidence of childhood leukaemia in the entire city was 2.5 times the United States national average during and after the time the wells were

pumping. For male subjects, the incidence in Eastern Woburn was 12.5 times that expected [13]. A positive statistical association between access to the water and the incidence of childhood leukaemia has been reported [11].

Residents of the East Woburn area receiving the highest concentration of water from wells G and H also complained of a variety of other health problems, including cardiac palpitations, frequent nausea, paresthesias, rashes and frequent infections. In May 1982 the families initiated a law suit against the companies thought to be responsible for the water contamination. In support of their complaints, detailed clinical and laboratory testing was done on the members of eight of the families in which a leukaemia case had occurred. All lived or had lived in the part of Woburn that received the highest concentration of contaminated water [11]. This testing took place 5 to 6 years after the closure of the wells, emphasis being placed on evaluation of the immune system, since a significant increase in pulmonary and urinary tract infections in children in East Woburn was observed when compared to children in West Woburn [11].

Materials and methods

Immunological, cardiological and general medical assessments were performed on 25 surviving family members of the leukaemia patients (Table 1). There was no significant history of exposure to organic solvents including benzene, halogenated hydrocarbons, heavy metals, herbicides and pesticides.

Immunological studies. White blood cell counts and lymphocyte levels were determined using conventional clinical laboratory histochemical staining techniques. Lymphocyte subpopulation analyses were carried out on Ficol-Hypaque separated blood lymphocyte preparations. Phenotypic analysis was performed by direct immunofluorescence with using a cytofluorograph (Ortho Diagnostics Raritan, NJ, USA) and fluorescein-conjugated monoclonal antibodies (Ortho Diagnostics) which included OKT3 recognising the CD3 antigen as a marker for T cells, OKT4 recognising the CD4 antigen on helper/inducer T cells and OKT8 recognising the CD8 antigen on suppressor/cytotoxic T cells [7]. Lymphocyte subpopulation levels were obtained by multiplying percentage composition values with total lymphocyte counts.

Total lymphocytes and lymphocyte subpopulations in the exposed population were analysed on three occasions. The samples were taken from 23 non-leukaemic adult subjects 5 years after well closure, tests were repeated 1 month and 17 months later. Control subjects for the first two series of tests were 30 healthy laboratory workers living in Boston.

In the third series, tests were carried out on coded samples and included as controls, 40 white residents of Boston randomly selected using standard area probability sampling techniques, matching with the exposed family members for age, sex and social habits. Total lymphocyte and lymphocyte subpopulation values in the two control groups run on three occasions did not differ significantly (data not shown) and were comparable to those used by reference clinical laboratories. Therefore the control data have been combined for comparison with the studies on the exposed population. The control values were within the ranges of those of reference clinical laboratories in the United States.

The phenotypic and functional profile of peripheral blood lymphocytes was compared to the randomly selected age- and sex-matched controls using an unpaired, one-tailed Student's *t*-test with the Welch modification. Additionally the lymphocyte subpopulations are depicted in the Figures using the Empirical Cumulative Distribution Method with observations spaced at intervals of 65 to 100 cells/mm³ for optimal formatting. At each observation point the percentage of subjects with values falling on or below that level is plotted.

Serological tests. Sera were taken from the 23 non-leukaemic adult subjects approximately 5 years after cessation of exposure and evaluated for auto-antibodies using indirect immunofluorescence methods. The panel included anti-smooth muscle, anti-ovarian, anti-nuclear, anti-thyroglobulin and anti-mitosomal antigens. Reference ranges were established in the clinical immunopathology laboratory which performed the tests and were 0–5% for each, in accordance with other studies [15].

General medical. General medical evaluations were performed through detailed histories, physical examinations, and serum chemistry tests to evaluate hepatic and renal function and lipid levels. Past medical records were also analysed.

Recurrent infections were defined as those responding to antibiotic therapy, and occurring more than twice per year over a 5-year period in an adult. Frequent or chronic sinusitis were defined as two episodes per year over 5-year period, responsive to antibiotics. In most cases this diagnosis was substantiated by medical records, and in some cases by findings on sinus x-rays. Chronic or frequent rhinitis was defined as appropriate symptoms continuing more than 3 months out of the year for 5 years with no seasonal component. Gastrointestinal (GI) complaints most often noted included chronic nausea with occasional vomiting and diarrhoea and were documented by history and medical records. In several cases upper and lower GI x-ray studies and sigmoidoscopic examinations were performed, and in no case was significant gross pathology demonstrated. Patients complaining of symptoms suspected to be cardiac in origin were given resting and exercise tolerance ECGs (ETT), 24-h Holter monitoring tests, and echocar-

diograms. Probability of clinically significant coronary artery disease was estimated by comparing serum lipid levels with the results of the ETT [20].

Results

Duration of well water exposure in relation to development of leukaemia

Table 1 lists the types of leukaemias in the families and duration of patient exposure to water from wells G and H. All of the patients with childhood leukaemias had been exposed in utero from the time of conception, and this exposure continued after birth. Most of these were acute lymphocytic leukaemia (ALL), 1 patient developed ALL after therapy for lymphosarcoma. Chronic myelogenous leukaemia occurred in 1 child and 1 adult. Of the 25 family members of patients A to H, 6 had also been exposed in utero to contaminated well water. Among the 25 subjects, 2 had other cancers, a basal cell carcinoma and a malignant melanoma, neither of these subjects had excessive sun exposure.

Immune profiles

Lymphocyte subpopulations of family members of leukaemic cases were analysed on three occasions after well closure (Table 2, Fig. 1). In the initial tests, family members showed a persistent lymphocytosis associated with an elevation in the absolute number of T cells (Fig. 1 A). These levels were statistically significantly higher than controls ($P < 0.01$). The CD4-positive cells (Fig. 1 B) and CD8-positive cells (Fig. 1 C) were also higher in the family members than controls. When compared to the controls, the family members had statistically significant ($P < 0.01$) lower helper/suppressor ratios (Table 2). Re-analysis 18 months later showed a statistically significant reduction in the total lymphocyte numbers, associated with a decrease in the T cells ($P < 0.01$ as compared to prior tests). This reduction was due predominantly to a significant loss of the CD8-positive cells ($P < 0.01$ as compared to prior tests) since the change in CD4-positive cells was statistically insignificant ($P < 0.11$, Table 2). The relative changes in lymphocyte populations caused an upward shift in the helper/suppressor ratio. The new ratio was not different from controls ($P < 0.56$) but significantly higher than the initial findings ($P < 0.11$). Such changes in helper/suppressor ratios are not seen in the normal population [12].

Auto-antibodies were seen in 48% (11/23) family members. Most had anti-nuclear antibodies (10/23), often with other auto-antibodies. No patient had positive anti-double-stranded DNA and no one with positive anti-thyroglobulin or anti-mitosomal antibodies (4/23) had abnormal thyroid function tests. Patients with leukaemia were not included in this analysis.

General medical

Of the 25 subjects 22 (88%) had a history of frequent or chronic sinusitis or rhinitis with no seasonal component. Sinus films on 3 patients with sinusitis showed thickened mucosa. Of the 25 subjects 13 (52%) had GI complaints which included chronic nausea and episodic diarrhoea and constipation, commonly described as the irritable bowel syndrome. These symptoms improved markedly with cessation of exposure to the contaminated well water

Table 1. Well water exposure of leukaemia patients and family members

Proband	Family member	Age at exposure to well water (years)	Tumour	Age at diagnosis (years)
A	Mother	29–43	ALL	4
	Child 1	3–17		
	Child 2	0–11		
	Child 3	0–13		
B	Father	25–27		
	Mother	19–22		
	Child 1	0–3		
C	Father	25–30	ALL	3
	Mother	15–30		
	Child 1	0–3		
D	Father	16–30	CML	31
	Mother	15–30		
	Child 1	0–8		
	Child 2	0–6		
E	Father	31–43	ALL	3
	Mother	28–41		
	Child 1	3–16		
	Child 2	0–12		
	Child 3	0–11		
	Child 4	0–19		
F	Mother	15–30	ALL	4
	Child 1	0–4		
	Child 2	0–7		
G	Father	29–44	Melanoma	47
	Mother	27–42	Basal cell carcinoma	45
	Child 1	0–15	CML	10
	Child 2	0–10		
H	Father	34–49	Lymphosarcoma/ALL	7
	Mother	31–46		
	Child 1	4–19		
	Child 2	3–18		
	Child 3	1–16		
	Child 4	0–9		

ALL, acute lymphocytic leukaemia; CML, chronic myelogenous leukaemia

Table 2. Peripheral blood lymphocyte populations in adult non-leukaemic family members and control subjects

Donor	Number of samples	Lymphocyte population (mean cells/mm ³ ± SD) expressing:			
		CD3 ^a	CD4 ^a	CD8 ^a	CD4/CD8 ratio
Controls	70	1562 ± 637	1002 ± 408	547 ± 260	1.94 ± 0.77
Family members tested on:					
5/84	25	2568 ± 1007 <i>P</i> < 0.01 ^b	1599 ± 787 <i>P</i> < 0.01	1123 ± 429 <i>P</i> < 0.01	1.49 ± 0.56 <i>P</i> 0.01
6/84	24	2655 ± 1159 <i>P</i> < 0.01	1587 ± 847 <i>P</i> < 0.01	1214 ± 579 <i>P</i> < 0.01	1.43 ± 0.61 <i>P</i> 0.01
11/85	20	1919 ± 618 <i>P</i> > 0.05	1295 ± 565 <i>P</i> 0.05	629 ± 199 <i>P</i> > 0.05	2.26 ± 1.11 <i>P</i> 0.56

^a Determined by reaction with monoclonal antibodies OKT3 (T cells), OKT4 (helper/inducer T cells) and OKT8 (suppressor/cytotoxic T cells)

^b Statistical analysis in comparison with control population, using an unpaired, one-tailed Student's *t*-test with the Welch modification
P values as compared to controls

but were still present more than 5 years later in all affected subjects.

In 13 of the 25 subjects, recurrent maculopapular rashes appeared within the 1st year of exposure. These occurred at least twice per year and lasted 2–4 weeks. They

were poorly responsive to steroids, were not associated with fever, and occurred most usually over extremities and face. No obvious cause could be found. In 3 cases, this was accompanied by anal pruritis which began with the rashes and resolved with them. In most subjects these rashes re-

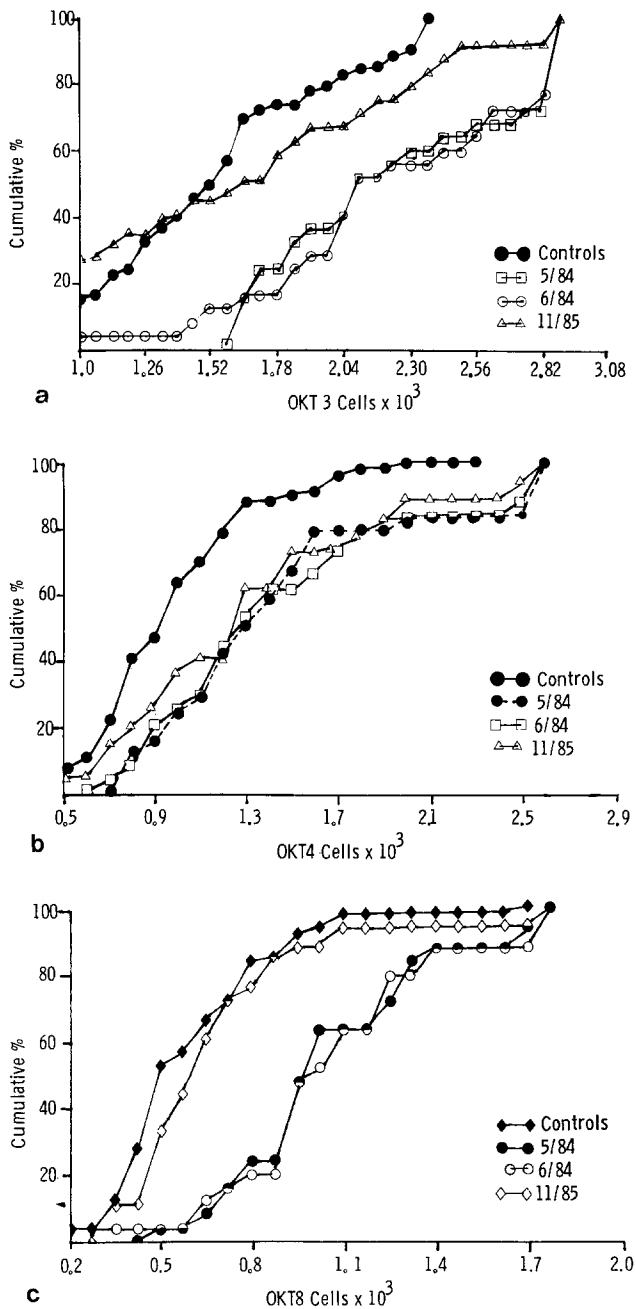


Fig. 1. Peripheral blood T lymphocytes and subpopulations in adult family members of leukaemia patients and control subjects, displayed in absolute numbers/ml serum as cumulative percentage of the population at various levels. Tests at three time points: May 1984 5 years after well closure, 1 month later (6/84) and 17 months later (11/85). (a) Peripheral blood T cells (CD3+). (b) Peripheral blood helper/inducer T cells (CD4+). (c) Peripheral blood suppressor/cytotoxic T cells (CD8+)

solved after the first 1 to 2 years of exposure, but in 4 cases, the rashes continued episodically past the cessation of exposure.

Cardiological

Of the adult subjects 14 complained of symptoms including unexplained rapid heart rate at rest, palpitations or near syncope and 11 patients were subjected to detailed cardiological testing including echocardiograms. Of the

11, 8 showed serious ventricular dysrhythmias, 7 had multifocal premature ventricular contractions and 6 of the patients required cardiac medication. Only 1 had mild mitral stenosis and this was haemodynamically insignificant. Calculations from the ETT, blood pressure, fasting blood sugars and cholesterol levels suggested that no subject had greater than a 0.1% chance of having clinically significant coronary artery disease [20].

Discussion

An increased incidence of leukaemia in children has been reported in residents of Woburn, Mass., compared with the national average in the United States, this being related to access to contaminated wells supplying domestic water [10, 11, 13]. Clinical and immunological studies were carried out on members of eight families in which a leukaemia case had occurred. The objective of this investigation was to ascertain whether abnormalities could be demonstrated which may reflect damage caused by exposure to contaminated water. These investigations revealed a clinical syndrome including gastroenteropathy, cardiomyopathy and dermatological and immunological abnormalities. Abnormalities in the neurological system have also been noted in other studies, (R. Feldman personal communication). Immunological damage was manifest by T lymphocytosis and by altered helper/suppressor (T4/T8) cell ratios which were unstable over time. There was also an increased incidence of auto-antibodies, particularly anti-nuclear antibodies. Associated with these changes, there was an increased incidence of infections and dermatological problems including recurrent rashes and pruritis ani.

The auxiliary well supplying water intermittently were contaminated with industrial solvents, TCE being the main volatile contaminant with lesser amounts of tetrachloroethylene and other chlorinated hydrocarbons [5]; TCE has been associated singly with several of the abnormalities noted in the individuals exposed to well water. It has been documented to produce cardiac arrhythmias [14, 17], encephalopathy and peripheral neuropathy especially trigeminal neuropathy [2, 3]. Industrial exposure leads to rashes [19] and TCE exposure in drinking water inhibits cell-mediated immunity and haematological progenitor cells in mice [16]. The dose necessary to produce these effects probably varies depending on the mode of exposure. The level of exposure seen in the Woburn families was lower than those reported as non-toxic in experimentally exposed populations [5, 9]. These investigations indicate the need for additional controlled studies to determine the incidence of symptoms in well water exposed compared to non-exposed populations.

Trichlorethylene is carcinogenic in animals [4, 6] and a suspect carcinogen in humans [8]. In animal studies its role as a leukaemogen is weak, however in the Woburn population there is a statistically significant increase of leukaemia in children exposed in utero to contaminated well water. Since neurological, cardiological and dermatological abnormalities have been previously seen in humans exposed to TCE, the Woburn population which has all of these abnormalities, probably represents a true syndrome of toxic exposure. The link between leukaemia and immunological abnormalities requires more critical evaluation. Familial propensity to leukaemia in children with parents having immunological abnormalities and a high incidence of auto-antibodies has been reported [1]. The aetiology of this

association could be either genetic or environmental, i.e., common exposure to agents which damage the immune system. This further emphasizes the need to evaluate the relationship between immune defects and exposure to carcinogens in controlled studies.

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