Immunological abnormalities 17 years after accidental exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin

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ABSTRACT Eighteen workers were reviewed 17 years after accidental exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). Clinical assessment showed that they were in good health. A study of several biochemical and immunological parameters in these subjects and in 15 carefully matched controls showed no difference in serum concentrations of hepatic enzymes between exposed workers and controls. Although mean serum concentrations of cholesterol and triglyceride were higher in exposed subjects than in controls, the results did not reach statistical significance. Antinuclear antibodies and immune complexes were detected significantly more frequently in the peripheral blood of workers exposed to dioxin. There was no significant difference between exposed workers and controls in the number of T lymphocytes, B lymphocytes, and helper and suppressor T cell counts in peripheral blood, but the number of natural killer cells identified by the monoclonal antibody Leu-7 was significantly higher in workers exposed to dioxin.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (dioxin) is a potent toxin produced as a byproduct in the manufacture of the herbicide 2,4,5 trichlorophenoxyacetic acid (2,4,5-T). Its toxic effects on various animals are well documented and include carcinogenesis,¹ teratogenesis,² hepatotoxicity,³ and immunological abnormalities, general debility, and death.⁴ There is, however, much controversy regarding its toxicity in man. Chloracne is the best known toxic effect of dioxin but many other effects have been reported including porphyria cutanea tarda and hepatotoxicity,⁵ neurological dysfunction,²⁻⁸ and asymptomatic hepatic enzyme induction.⁹⁻¹⁰ Although there is some evidence linking 2,4,5-T and exposure to dioxin with an increased risk of soft tissue sarcoma,¹¹ lymphoma,¹² and atherogenesis,^{5,13} these associations are not proved.¹⁴ Immunological abnormalities have been described in animals but there is relatively little information about the immunological consequences of dioxin exposure in man. We have recently studied 18 subjects who were exposed to dioxin as a result of an industrial accident in 1968. We describe the results of biochemical and immunological investigations performed on these subjects and 15 carefully matched controls.

Subjects

Group 1—Eighteen workers exposed to dioxin as a result of an industrial accident during the manufacture

of 2,4,5-T. Three were in the building at the time of the accident, nine were engaged in clearing up after the explosion, and 16 helped wash and repaint the building. Eight suffered from chloracne. Thirteen had worked in the relevant building regularly before the accident and seven worked regularly in the new building afterwards. All were healthy at the time they were seen, none having symptoms of a recent viral infection. None of the subjects studied had symptomatic ischaemic heart disease or evidence of neoplastic disease. Two were taking regular medication in the form of ranitidine (1 subject) and metformin (1 subject). This group of subjects has been included in previous studies.^{9,13,15}

Group 2—Fifteen controls were recruited from the portering staff and the department of estate management at this institution. Many of the porters had previously worked in local industry for many years before their current employment but none had knowingly been exposed to toxic chemicals. Controls were matched for age, sex, per cent of ideal body weight,¹⁶ social class, alcohol consumption, and smoking habit (alcohol consumption is expressed in units; one unit was defined as $\frac{1}{2}$ a pint of beer, one glass of wine, or one measure of spirit). All were healthy at the time they were seen and none was taking any medication.

Methods

All subjects who had been exposed to dioxin underwent a complete clinical assessment and a resting

ECG. Blood was drawn from exposed subjects and controls after an overnight (12 hour) fast. Investigations performed included:

- (1) full blood count and ESR;
- (2) serum creatinine, γ -glutamyl transferase, alanine aminotransferase, calcium, albumin, and globulin (SMAC II, Technicon, New York);
- (3) plasma glucose;
- (4) serum thyroxine, triiodothyronine, and thyroid stimulating hormone;
- (5) serum cholesterol, triglyceride, and high density lipoprotein cholesterol (HDL cholesterol), measured using Boehringer reagents on a Cobas Bio centrifugal analyser (HDL cholesterol after precipitation of other lipoproteins with dextran sulphate and magnesium);

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(6) serum IgG, IgA, IgM, were measured using an Encore centrifugal analyser (Baker Instruments, Pleasantville, NY), serum IgD by single radial immunodiffusion, and serum IgE by Phadebas PRIST (Pharmacia, Milton Keynes);

(7) antinuclear antibodies (ANA) were measured by indirect immunofluorescence. Both rat liver and Hep2 cells (Kallestad UK Ltd, Brill, Bucks, UK) were used as substrate. Antibodies to extractable nuclear antigen were detected by Ouchterlony double diffusion technique;

(8) immune complexes were shown by precipitation with polyethylene glycol;

(9) lymphocyte separation and enumeration: lymphocytes were separated from heparinised peripheral whole blood by standard Ficoll-Hypaque (Pharmacia, Milton Keynes) density gradient centrifugation. T lymphocyte subpopulations and natural killer (NK) cells were measured by indirect immunofluorescence with monoclonal antibodies Leu-1, Leu-2a, Leu-3a, Leu-7 (Becton Dickinson, Mountain View, California) and fluorescein isothiocyanate conjugated (FITC) Fab'2 goat antimouse IgG. B lymphocytes were counted using FITC antihuman immunoglobulin.

(10) lymphocyte proliferation was assessed by incorporation of tritiated thymidine into cells stimulated by purified Phytohaemagglutinin A (Wellcome, Beckenham, Kent) at doses of 0.1 $\mu\text{g}/\mu\text{l}$, 0.01 $\mu\text{g}/\mu\text{l}$, and 0.001 $\mu\text{g}/\mu\text{l}$.

Statistical analysis was by Student's *t* test, Fisher's exact test, and χ^2 analysis.

Results

As may be seen from table 1, the individuals who had been exposed to dioxin did not differ significantly from controls with respect to age, per cent of ideal body weight, alcohol consumption, or smoking habit; they were also matched for social class. Serum concentrations of hepatic enzymes did not differ significantly in

Table 1 Characteristics of study patients

	Group 1 (n = 18)	Group 2 (n = 15)	p
Age (years)*	50.7 (14.3)	48.7 (9.8)	NS
Percentage of ideal body weight*	112.2 (13.0)	111.7 (12.4)	NS
Units of alcohol a week (mean)	21	20	NS
No of subjects who smoke	10	8	NS

*Mean (standard deviation).

the two groups. Although mean serum cholesterol and mean serum triglyceride were slightly higher in the group exposed to dioxin, the difference did not reach statistical significance (mean (SD) cholesterol 6.8 (1.1) v 6.3 (1.2); mean (SD) triglyceride 2.1 (0.6) v 1.8 (0.8); 0.1 > p > 0.05 for both). Equally, the level of HDL cholesterol in the dioxin exposed workers was not significantly lower than in controls (mean (SD) 1.2 (0.3) v 1.4 (0.3); 0.1 > p > 0.05). None of the subjects studied had biochemical evidence of thyroid dysfunction.

There was no significant difference in the levels of immunoglobulins G, A, M, D, and E between the two groups. ANA was detected significantly more frequently in workers exposed to dioxin than in controls when Hep2 cells were used as substrate (table 2) (p < 0.01) but not when rat liver was used. The staining pattern observed with Hep2 cells was predominantly of diffuse grainy type (diffuse grainy in eight subjects, nucleoli in two subjects). Extractable nuclear antigen was not detected in any of the subjects studied. Immune complexes were detected significantly more frequently in individuals exposed to dioxin than in controls (p < 0.05); however, they were usually present at low titre. Total lymphocyte count, B cell count, T cell count, and helper and suppressor T cell counts did not differ significantly in the two groups (table 3). The number of NK cells identified by Leu-7 was, however, appreciably raised in the workers exposed to dioxin (p < 0.002). The lymphocyte proliferative response to phytohaemagglutinin A was not significantly altered in the workers exposed to dioxin. A helper/suppressor ratio below 1.5 occurred in nine individuals who had been exposed to dioxin and three controls. This was not, however, significant at the 0.05 level.

Table 2 Antinuclear antibodies and immune complexes in subjects exposed to dioxin and controls

	Group 1 (n = 18)	Group 2 (n = 15)	p
No of subjects ANA positive (rat liver)	5	0	NS
No of subjects ANA positive (Hep2 cells)	8	0	<0.01
No of subjects with immune complexes	11	3	<0.05

Table 3 Lymphocyte subpopulations

	Group 1 (n = 18)	Group 2 (n = 15)	p
Total lymphocytes ($\times 10^9/l$)*	1.98 (0.84)	2.02 (0.47)	NS
B Cells ($\times 10^9/l$)*	0.21 (0.11)	0.16 (0.08)	NS
T Cells ($\times 10^9/l$)*	1.60 (0.44)	1.68 (0.47)	NS
T-helper ($\times 10^9/l$)*	0.95 (0.34)	1.04 (0.29)	NS
T-suppressor ($\times 10^9/l$)*	0.63 (0.28)	0.59 (0.23)	NS
Natural killer ($\times 10^9/l$) (Leu-7)*	0.40 (0.21)	0.19 (0.15)	<0.002

*Mean (SD).

Discussion

In animals dioxin is known to induce several immunological abnormalities including thymic atrophy¹⁷ and T lymphocyte depletion in peripheral lymphoid tissue.¹⁸ T cell abnormalities include depressed delayed hypersensitivity skin responses,¹⁸ reduced lymphoproliferative responses to various mitogens,^{18,19} and depressed generation of cytotoxic T lymphocytes.²⁰ B lymphocytes may be suppressed in animals exposed to higher doses of dioxin²¹ and humoral responses may also be impaired.²²

There is relatively little information about immune abnormalities after exposure to dioxin in man. Reggiani reported normal percentages of T and B lymphocytes and normal responses to mitogens in children exposed to dioxin at Seveso.²³ Knutsen *et al* studied subjects exposed to dioxin contaminated soil in Missouri and found no difference in T cell subsets between those at high risk for dioxin exposure and those at low risk.²⁴ They also found no difference in lymphoproliferative responses to four mitogens. Hoffman *et al* studied another group of subjects exposed to dioxin in the environment.²⁵ They found that exposed subjects had a significantly reduced percentage of helper T lymphocytes but that there was no significant difference in the absolute numbers of helper and suppressor T lymphocytes. Our findings are in general agreement with those described above in that we found that the number of T cells, B cells, and helper and suppressor T cells were not significantly different in the group exposed to dioxin than in the controls. Our findings also agree with those of Reggiani²³ in that individuals exposed to dioxin had similar serum immunoglobulin concentrations to controls.

Immune complexes, however, occurred significantly more frequently in subjects exposed to dioxin. The complexes were generally present at low titre but we think that this warrants further investigation, perhaps in subjects with more recent exposure to dioxin. ANA was also detected more frequently in subjects exposed to dioxin, when Hep2 cells were used as substrate. The specificity of this antibody is uncertain but it would not seem to be directed against a saline extractable antigen such as those detected by the Ouchterlony

system. While the significance of this finding in the inherently more sensitive Hep2 cell system is not clear, it may reflect prior tissue damage and cellular destruction in the exposed population.

We have shown for the first time an increased number of circulating NK cells in subjects exposed to dioxin, using the monoclonal antibody Leu-7. Leu-7 has been shown to be a marker for most NK cells,²⁶ although more recently Lanier *et al* have shown that Leu-11a detects more cells with a high degree of NK activity than Leu-7.²⁷ We have used the antibody Leu-11a in a subgroup of our patients and have obtained results similar to those obtained with Leu-7. NK cells are believed to contribute to defence against viruses and tumours.^{28,29} When abnormalities related to NK cells have been reported, the usual finding is of impaired NK function and this has been shown in subjects with various solid tumours.^{28,29} Increased NK activity has been reported in people with viral illnesses.²⁹ There is, however, little work on the number of NK cells in peripheral blood. A reduced number of NK cells as delineated by Leu-7 has been described in large cell lymphoma where there is active disease³⁰ and reduced NK function has also been noted.³¹ Behcet's disease³² and NK proliferative disease³³ have been reported to increase the number of NK cells in man and viral infection has been shown to increase the number of NK cells in mice.³⁴ The number of NK cells as defined by monoclonal antibody may, however, not reflect NK function.^{32,35} Although there are, to our knowledge, no studies of NK function in man, Mantovani *et al* reported NK activity per cell in mice to be normal after the acute administration of dioxin.³⁶ They also found that the number of splenocytes recovered was significantly reduced, suggesting a reduction in the number of NK cells. Nevertheless, experimental evidence relating to mouse spleen NK cells some weeks after exposure to dioxin is not strictly comparable with the effect of previous exposure to dioxin on NK cells in human peripheral blood.

Statistically significant increases in the serum cholesterol and triglyceride concentrations of workers exposed to dioxin have been reported.^{13,15,37} Although mean serum cholesterol and triglyceride concentrations were higher in our subjects who had been exposed to dioxin than controls, the differences were not statistically significant. Our results were, however, similar to those obtained by Martin in a larger number of subjects.¹⁵ Other authors have reported lipid levels in subjects exposed to dioxin to be comparable with those in controls.^{9,10}

Previous work has shown increased serum concentrations of γ -glutamyl transferase and alanine aminotransferase in subjects exposed to dioxin^{9,10} but we found none. Our findings may reflect the long interval since last exposure to dioxin.

We have thus demonstrated several immunological differences between subjects with pronounced

exposure to dioxin 17 years before our study and controls. It is not entirely surprising that changes have been noted so long after the original explosion, as chloracne may persist for at least ten years after exposure to dioxin^{5,9} and hyperlipidaemia has been noted ten years after exposure to dioxin.¹⁵ The significance of the increased number of circulating NK cells is uncertain. Some authors have noted an increased tendency to infections in subjects exposed to substantial quantities of dioxin,³⁸ although this has usually been described soon after exposure. If exposure to dioxin is associated with an increased risk of neoplastic disease the number and function of NK cells may be important in its pathogenesis. Alternatively, changes in the number of NK cells may show activation of the body's defences. We think that further studies of NK cell number and function in subjects exposed to dioxin are indicated.

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