

Pulmonary epithelial response in the rat lung to instilled Montserrat respirable dusts and their major mineral components

D G Housley, K A Bérubé, T P Jones, S Anderson, F D Pooley, R J Richards

Occup Environ Med 2002;**59**:466–472

See end of article for authors' affiliations

Correspondence to:
Professor Roy Richards,
School of Biosciences,
Cardiff University, PO Box
911, Museum Avenue,
Cardiff CF1 3US, Wales,
UK;
RichardsRJ@Cardiff.ac.uk

Accepted
13 February 2002

Background: The Soufriere Hills, a stratovolcano on Montserrat, started erupting in July 1995, producing volcanic ash, both from dome collapse pyroclastic flows and phreatic explosions. The eruptions/ash resuspension result in high concentrations of suspended particulate matter in the atmosphere, which includes cristobalite, a mineral implicated in respiratory disorders.

Aims: To conduct toxicological studies on characterised samples of ash, together with major components of the dust mixture (anorthite, cristobalite), and a bioreactive mineral control (DQ12 quartz).

Methods: Rats were challenged with a single mass (1 mg) dose of particles via intratracheal instillation and groups sacrificed at one, three, and nine weeks. Acute bioreactivity of the particles was assessed by increases in lung permeability and inflammation, changes in epithelial cell markers, and increase in the size of bronchothoracic lymph nodes.

Results: Data indicated that respirable ash derived from pyroclastic flows (20.1% cristobalite) or phreatic explosion (8.6% cristobalite) had minimal bioreactivity in the lung. Anorthite showed low bioreactivity, in contrast to pure cristobalite, which showed progressive increases in lung damage.

Conclusion: Results suggests that either the percentage mass of cristobalite particles present in Montserrat ash was not sufficient as a catalyst in the lung environment, or its surface reactivity was masked by the non-reactive volcanic glass components during the process of ash formation.

Soufriere Hills is a moderate sized calcalkaline volcano situated on the southeastern part of the Lesser Antilles island of Montserrat. Prior to the present eruption, the last known eruption occurred AD 1646 ± 54 years, although there have been a number of non-eruptive volcano related seismic events, including 1897–98, 1933–36, and 1966–67.^{1,2} The latest eruption began on 18 July 1995, and continues to be active to the present (2002). The first two months of activity involved the formation of vents producing steam, non-juvenile ash, and blocks, and the generation of some mudflows. Around 15 November the first definite lava was recorded at the surface from the 18 July crater. A new dome started developing in late February 1996, and a collapse from this dome resulted in the first pyroclastic flow. In the ensuing six months there were many pyroclastic flows down the eastern slope of the volcano. August and September 1996 saw intense volcanic activity, culminating in an explosion on 17–18 September, which covered the nearby town of Plymouth in a 600 000 ton layer. The magma is a typical Lesser Antilles andesite with a large crystalline component (69–80%), and with 58–60% SiO₂. The crystalline silica in the ash is mostly cristobalite, with the ash generated from dome collapse pyroclastic flows ranging from 10–24% cristobalite, whereas the explosive eruption ash-falls have a lower range of 3–6% cristobalite.³

Concern has been expressed that local residents or those on neighbouring islands who are exposed to high levels of ash containing cristobalite may develop respiratory disorders.^{3,4} Those at risk include workers involved in clean up operations or residents who wish to reoccupy currently off-limits areas after the cessation of the eruption.

The objective of the study was to compare the bioreactivity of well characterised respirable samples of ash from the volcano together with the major dust components of the mixture and appropriate bioactive control samples. Rats were challenged with a single mass (1 mg) dose of particles via intratracheal instillation; groups were sacrificed at three time

points (up to nine weeks). The dose of 1 mg of dust delivered to rats by intratracheal instillation was chosen because investigations with other dusts have indicated that this dose can induce inflammatory changes in the lung and translocate the pulmonary epithelium.^{5,6} In order to achieve this deposition by inhalation, clouds of cristobalite dust at a concentration of 39 mg/m³ for eight days are required.⁷ Although direct comparisons between humans and rats is impossible, eruption concentrations of volcanic ash on Montserrat of 0.6 mg/m³ have been reported.⁸

Acute bioreactivity of the particles was determined by the occurrence of pulmonary inflammation (increases in lavage cells), increases in lung permeability (elevation in lung/body weight and bronchoalveolar lavage acellular protein), changes in epithelial cell markers (pulmonary surfactant, γ glutamyl transpeptidase, rTl₄₀) and increase in size of bronchothoracic lymph nodes. By including time in the experiments (one, three, and nine weeks post-instillation), it was possible to determine whether damage was immediate, transient with recovery, or progressive, leading to the risk of more severe lung disease.

METHODS AND MATERIALS

Particulate samples

Two volcanic ash samples were collected from Montserrat by Prof. R Maynard, CBE (DOH, UK), and one from the nearby island of Antigua by Mr R Williamson. Montserrat 1 was collected on 21 September 1997, and was ash generated by a pyroclastic flow. Montserrat 2 was collected on 24 September 1997 and was ash from a phreatic explosion. Volcanic dust was

Abbreviations: GGT, γ glutamyltransferase; IA, image analysis; PMN, polymorphonuclear leucocyte; TEM, transmission electron microscopy; XRD, x ray diffraction; XRF, x ray fluorescence

Table 1 The major elements of volcanic ashes and control minerals (as % oxide by weight) as determined by XRF

Element	Cris/obs	Anorthite	Labradorite	Montserrat 1	Montserrat 2	Antigua
SiO ₂	75.69	57.48	54.82	64.83	60.60	58.46
Al ₂ O ₃	12.94	25.69	27.64	15.96	18.51	18.70
Fe ₂ O ₃	0.94	0.39	0.41	6.00	6.65	7.34
MnO	0.06	0.01	0.01	0.15	0.16	0.16
MgO	0.03	0.07	0.12	2.30	2.62	2.78
CaO	0.73	7.99	10.23	5.50	7.33	8.51
Na ₂ O	3.06	5.19	4.17	2.50	2.65	2.39
K ₂ O	4.45	0.56	0.51	1.04	0.82	0.71
TiO ₂	0.06	0.10	0.12	0.51	0.56	0.66
P ₂ O ₅	0	0.01	0.02	0.14	0.13	0.12

*Cristobalite/obsidian.

collected on Antigua on 12 October 1997; the ash was generated from a strong explosion on Montserrat the previous day, after which the cloud rose to a height of 22 000 feet, entered the airsteam, and was carried to Antigua, 34 miles to the east in less than two hours. Samples containing particles in the respirable size range (99% <3 µm diameter) were prepared from the bulk collections by centrifugal sedimentation; each was examined for size distribution by transmission electron microscopy (TEM) and image analysis (IA),⁹ and crystalline mineralogy was determined using x ray diffraction (XRD).

A selection of mineral control samples were chosen, based on the mineralogy and geochemistry of the Montserrat ash (see results). The selection included two cristobalites and two feldspars. A sample of pure cristobalite was taken from a 20 year old sample, which was probably manufactured by the heating and quenching of an opaline material, SiO₂.nH₂O. The cristobalite mineralogy was confirmed by XRD on both a parent sample and one subjected to acid (1M HCl) washing, which probably removes the 30 nm "amorphous" surface layer of silanol groups that are associated in the reduction of biological activity of aged samples.¹⁰ The acid washed sample was subsequently washed with four changes of HPLC grade water. The second cristobalite sample, cristobalite/obsidian, was a natural sample of cristobalite from a volcanic lava flow from the United States. The rock was obtained from a commercial dealer (Northern Geological Supplies, Richard Taylor Minerals, UK), and consisted of intercalated dull grey cristobalite (~40%) and shiny black obsidian (volcanic glass, ~60%). The cristobalite was confirmed by XRD; volcanic glass does not give an XRD signal. As it was impossible to separate the two components, the mineral control included both components.

A further control mineral was a sample of the natural mineral anorthite (CaAl₂Si₂O₈), in the form of the igneous rock

anorthosite (consisting of more than 90% anorthite). Anorthite was chosen as it represents plagioclase feldspar from the most calcic extreme seen in the range of the Montserrat ash. A second control feldspar was a sample of the natural mineral labradorite ((Ca.Na)Al₂Si₂O₈). Plagioclase feldspar is a solid-solution series between the two end members anorthite (CaAl₂Si₂O₈) and albite (NaAlSi₃O₈), with labradorite (An₅₀₋₇₀) sitting towards the most sodic end of the range seen in the Montserrat ash. The two feldspar controls thus represent the calcic and sodic extremes seen in the Montserrat feldspars. Cristobalite/obsidian, anorthite, and labradorite were analysed by x ray fluorescence (XRF) using standard routines as described elsewhere.¹¹ These control samples were crushed to a coarse grit and then powdered in a tungsten steel mill to produce respirable particles (99% <3 µm diameter), the size distribution of which was determined by TEM/IA. The crystalline silica, alpha quartz, DQ12 (Dorentrop mine, Germany) served as a positive bioreactive control dust and 0.15M NaCl, used as a delivery vehicle, served as a sham control.

Particle instillation

Male, specific pathogen free, CD1 Sprague-Dawley rats (Charles River UK Ltd; initially 176–200 g body weight) were used throughout the project. They were acclimated for one week in their own holding unit and in isolation from other animal stocks. They were housed in wire bottomed cages in controlled temperature and lighting environment, and fed rat chow pellet food and water ad libitum.

Dusts were prepared at 2.5 mg/ml in 0.15M NaCl (20°C) and maintained in an ultrasonicated bath prior to being administered as an intratracheal instillate using a non-surgical technique.⁵ Animals were anaesthetised in an atmosphere of halothane prior to the instillation of 400 µl (1 mg) of the above. For each dust, animals (n = 5) were sacrificed at one,

Table 2 Particle size distribution for Montserrat and Antigua respirable dusts in comparison with their major mineral components

ESD	Mon 1	Mon 2	Antigua	Ano	Lab	Cris/obs	Cris	DQ12
0.1	0	0	0	0	0	0	0	0
0.15	12	12	12	28	10	20	10	5
0.2	14	22	6	9	10	11	6	8
0.3	22	25	18	17	23	17	7	27
0.4	11	11	14	12	17	8	6	15
0.5	7	7	9	9	10	4	8	11
0.75	9	8	15	10	11	12	22	16
1	5	4	8	6	5	7	13	6
2	11	5	14	6	8	16	18	11
3	5	3	3	2	3	4	5	0
4	1	1	1	1	2	1	2	0
5	1	0	0	1	1	0	1	0
More	0	1	0	1	1	1	1	0

Results expressed as percentages.

Mon 1, Montserrat 1; Mon 2, Montserrat 2; Ano, anorthite; Lab, labradorite; Cris/obs, cristobalite/obsidian.

ESD, equivalent spherical diameter.

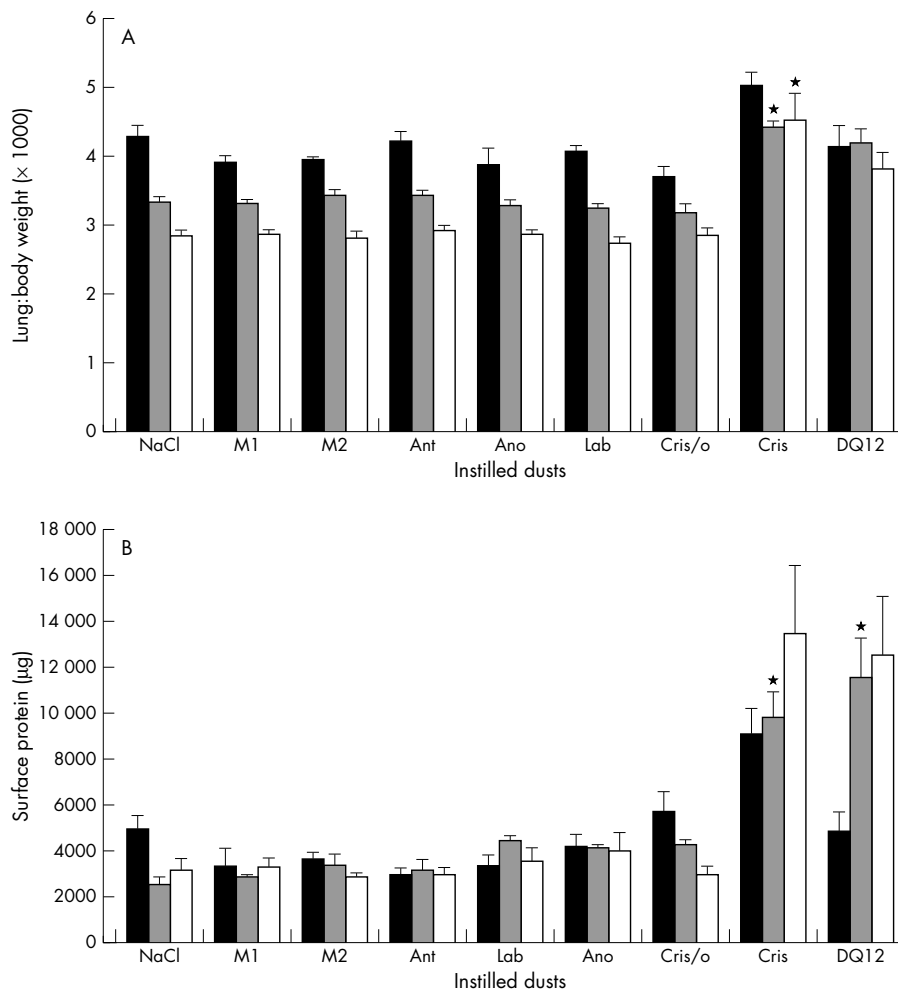


Figure 1 Changes in (A) mean lung:body weight (\pm SE) (ANOVA for differences between groups: $F = 6.43$, $p < 0.0001$; $F = 16.02$, $p < 0.0001$; $F = 7.97$, $p < 0.0001$, for weeks 1, 3, and 9 respectively); and (B) acellular lung surface protein with time after instillation with volcanic dusts and their mineral controls ($F = 6.48$, $p < 0.0001$; $F = 14.46$, $p < 0.0001$; $F = 5.51$, $p < 0.0001$, for weeks 1, 3, and 9 respectively). *Denotes significantly different ($p < 0.05$) from sham treated control (NaCl only). Black bars = 3 weeks, grey bars = 6 weeks, white bars = 9 weeks post-instillation. M1, Montserrat 1; M2, Montserrat 2; Ant, Antigua; Ano, anorthite; lab, labradorite; Cris/O, cristobalite/obsidian; Cris, cristobalite; DQ12, quartz.

three, and nine weeks after instillation. No animal showed signs of distress or failed to gain weight at any time during the course of the experiment. At the end of each time point, animals were anaesthetised with halothane prior to being given a lethal dose of Sagatal (pentobarbitone, 60 mg/ml). Animals were weighed, exsanguinated, and the lungs perfused via the pulmonary artery, prior to being removed en bloc and weighed. Lymph nodes were removed and photographed. Lungs were lavaged with 50 ml (5×10 ml) of 0.15M NaCl.

Recovered lavage fluids were centrifuged at 300 g for 10 minutes to remove free cells. The cell pellet was resuspended and total cell numbers were determined by haemocytometer counts. Supernatant samples were stored at -70°C until analysis. The remainder of the 300 g supernatants was pooled for each group for isolation of pulmonary surfactant.³

Biochemical analysis

Total acellular lavage protein (300 g supernatant) was assayed by the method of Lowry and colleagues,¹² using bovine serum

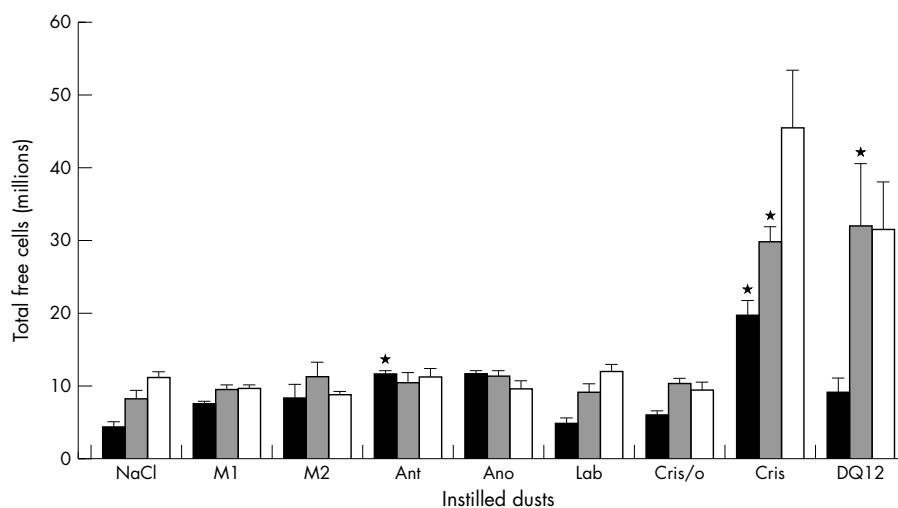


Figure 2 Changes in numbers of free cells in lavage fluids with time after instillation with volcanic dusts and their mineral controls (ANOVA for differences between groups: $F = 11.92$, $p < 0.0001$; $F = 11.02$, $p < 0.0001$; $F = 7.92$, $p < 0.0001$, for weeks 1, 3, and 9 respectively). *Denotes significantly different ($p < 0.05$) from sham treated control (NaCl only). Data bars and abbreviations for dusts are as described in fig 1.

albumin (Sigma, UK) as a standard. γ Glutamyl transferase (GGT), a cell surface marker for type 2 and Clara cells,¹³ was assayed in the 300 g supernatant by Sigma kit method 545A. The type I cell marker, rTl₄₀ was assayed in the 300 g supernatant by dot blot.¹⁴ Briefly, lavage samples were diluted to 10 μ g/ml total protein. Each sample was run in duplicate with 100 μ l per well being blotted onto Hybond-C (Amersham, UK) membrane, using a Bio-Rad dot blotter. Whole untreated adult lung homogenate diluted to a range of 0–1 μ g/ml total protein was used as standard. The membrane was developed using standard Western blot protocols, with sheep antirabbit HRP conjugate (Amersham, UK) as secondary antibody and visualised using ECL (Amersham, UK). The intensity of developed dots was measured by absorbance (540 nm) and compared to controls. For data analysis, 1 μ g of rTl₄₀ was equivalent to the rTl₄₀ concentration of lung (wet weight) of adult rat lung homogenate.

Statistical analysis

All data were analysed using MINITAB (Minitab Inc., Pennsylvania) and the Intercooled STATA statistical package.¹⁵ All lavage data are expressed as total/rat. Differences between means used a two sample *t* test. A Bonferroni multiple comparison of data, at each time point, from sham and dust treated animals was analysed using one way ANOVA (STATA command: oneway), while a modified one way ANOVA by rank (Kruskal–Wallis) test (STATA command: kwallis2) allowed the control group to be specified. Statistical significance was accepted at $p < 0.05$. Analysis showed that for all parameters studied there were no significant differences between values obtained with acid washed cristobalite and the untreated parent cristobalite, thus data for these two groups were pooled ($n = 10$); $n = 5$ for all other groups.

RESULTS

Physicochemical analysis of particulate samples

A combination of XRD, bulk analysis, and TEM/electron probe *x* ray microanalysis (data not shown) confirmed that Montserrat 1 (pyroclastic flow), Montserrat 2 (phreatic explosion), and Antigua dust contained 20.1%, 8.6%, and 4.7% cristobalite respectively. XRF confirmed that the silica content decreased Montserrat 1 > Montserrat 2 > Antigua, and provided elemental analysis of the control samples of volcanic glass (cristobalite/obsidian) and the feldspars (anorthite, labradorite) (table 1). The analytical data are very similar to that reported recently by Baxter and colleagues,³ and formerly by Rea.¹

Table 2 shows size distributions of the prepared respirable samples of volcanic ashes and control materials. These indicate that ultrafine particles (<100 nm) are not detected despite sufficient resolution with the technique, and that coarse particles (>5 μ m) are extremely rare (<1%). Thus, all the samples contain fine particles, of which the majority (>93% by number) range between 150 nm and 2 μ m. Nevertheless, some differences in size distribution are noted. In particular, the prepared cristobalite had large numbers of particles in the size range 0.75–2 μ m and thus less particles 0.5 μ m or below (37%) than all of the other materials investigated (60–77% are 0.5 μ m or below).

Biochemical and cellular analysis

A significant increase in lung:body weight (fig 1A) provides one measure of oedema, and hence, increases in epithelial permeability. A more sensitive measurement of permeability is detected with an increase in lavage acellular (lung surface) protein (fig 1B). Instillation of the volcanic ashes or feldspars causes no significant changes in lung:body weight compared with sham treated rats at any timepoint (fig 1A). Similarly, treatment with the same dusts produces few significant increases in lung surface protein (with the exception of the

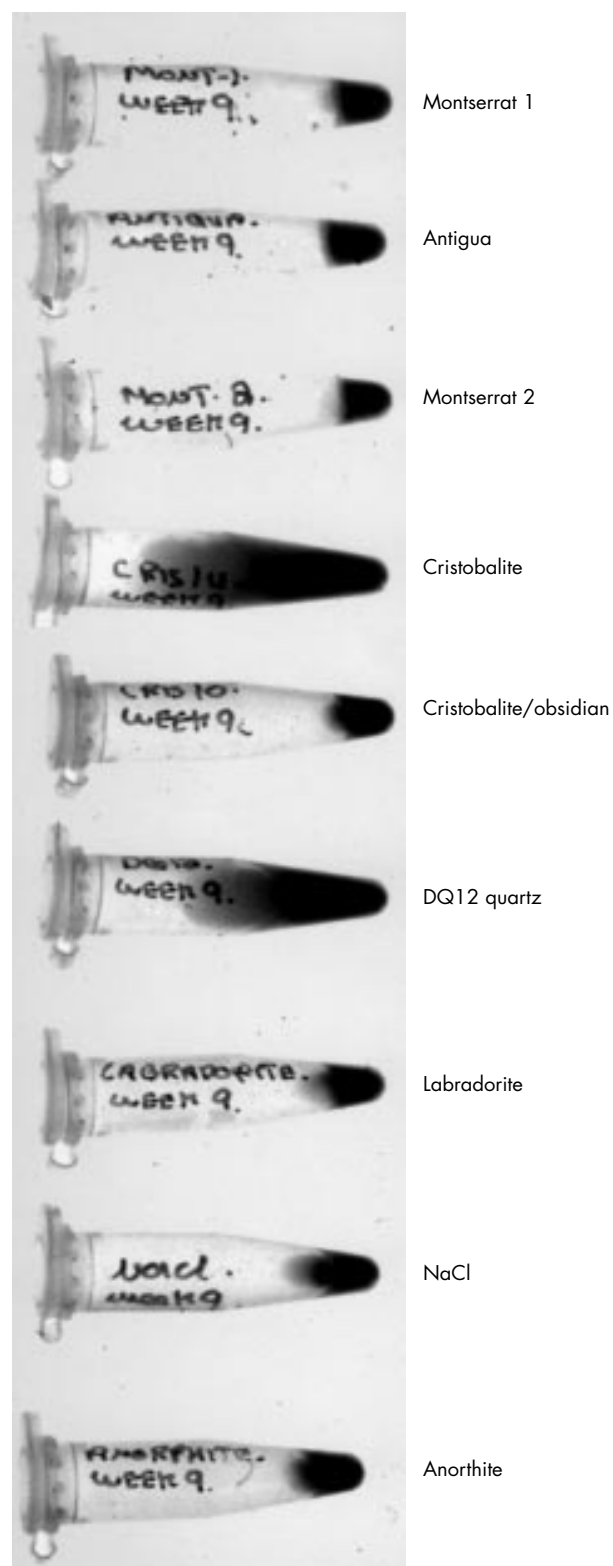


Figure 3 Free cell and surface debris (300 g pellets from lavage) in pooled samples from five animals in each group, nine weeks post-instillation of volcanic dusts and their mineral controls.

feldspars at three weeks) (fig 1B). In direct contrast, cristobalite treatment produces significant elevations in lung:body weight ratio ($\chi^2 = 73.3$; $p < 0.0001$) as well as surface protein, effects which are also noted with DQ12 quartz (except at one week). The cristobalite/obsidian mixture significantly lowers lung:body weight ratio at one week and mimics the elevation

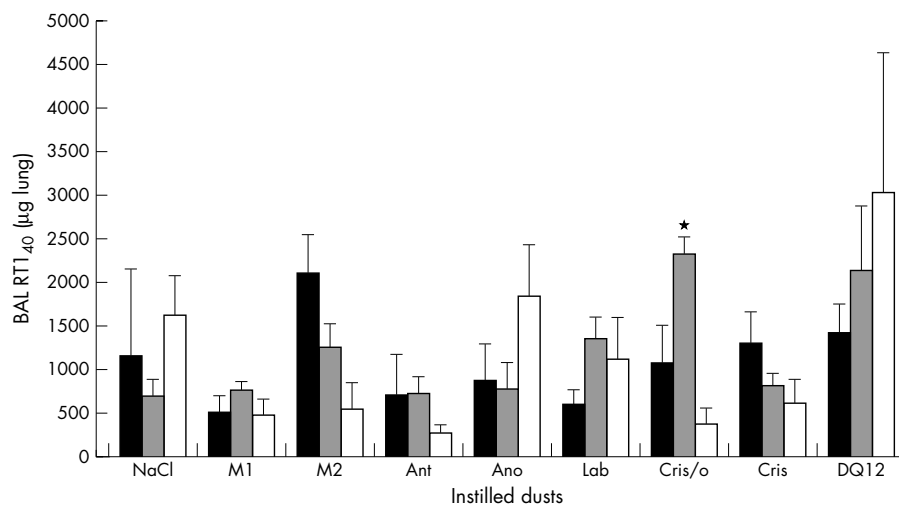


Figure 4 Changes in rT1₄₀ with time after instillation with volcanic dusts and their mineral controls (ANOVA for differences between groups: $F = 1.16$, NS; $F = 4.26$, $p < 0.001$; $F = 2.24$, $p < 0.05$, for weeks 1, 3, and 9 respectively). *Denotes significantly different ($p < 0.05$) from sham treated control (NaCl only). Data bars and abbreviations for dusts are as described in fig 1.

in surface protein at three weeks, as observed with the feldspars.

The free cells derived from lung lavage were counted and expressed as total cells (millions) recovered (fig 2). Seven days after instillation, animals treated with Antigua and cristobalite had significantly increased free cell numbers (11.5 (95% CI: 10.3 to 12.7) and 19.4 (14.6 to 24.3) $\times 10^6$ total free cells respectively) compared to control animals (4.3 (1.6 to 6.9); $F = 11.92$; $p < 0.0001$). Differential staining of cytopins of the cells indicated that the majority were macrophages. By week 3 only cristobalite and DQ12 treated animals (29.7 (25.1 to 34.2) and 31.6 (7.7 to 55.5) $\times 10^6$ total free cells respectively) had higher means than sham controls (8.2 (5.0 to 11.4) $\times 10^6$ free cells; $F = 11.02$, $p < 0.0001$). Free cell numbers remained significantly increased in cristobalite treated animals at nine weeks ($F = 7.92$, $p < 0.0001$). In these dust treated animals only, polymorphonuclear leucocytes (PMN), distinguished by differential staining of cytopins, accounted for 40–60% of the free cell count. In all other dust treated animals PMN account for <2% of the total population. A visual representation of the cells/debris present in the lavage 300 g pellets (pooled samples from five animals per group) at nine weeks post-instillation shows the similarity of surface reaction to volcanic ashes and feldspars, in contrast to the enhanced response seen with the bioreactive quartz and cristobalite (fig 3).

Early lung damage, induced by bioreactive substances, often occurs to the type 1 epithelial cell which covers most of the alveolar surface.¹⁶ A surface marker protein, rT1₄₀, has been reported for this cell type,¹⁷ and other workers have shown that this is increased at the lung surface during pulmonary damage.¹⁴ Highly variable levels of rT1₄₀ are found in the acellular fraction of lung lavage samples (fig 4). As a result, significant differences between the mean values of dust and sham treated animals are rare. A notable exception was seen with cristobalite/obsidian (increase at week 3; $F = 4.26$, $p < 0.05$). Interestingly, high levels of rT1₄₀ (not significant) are found at three and nine weeks with DQ12 quartz in direct contrast to results observed with the other crystalline silicon dioxide, cristobalite.

Change in lung epithelium following dust exposure was assessed with two further markers in the lavage sample, pulmonary surfactant (table 3) and GGT (fig 5). Pulmonary surfactant is a secretory product of alveolar type 2 cells and is significantly accumulated following exposure to bioreactive dusts such as quartz,¹⁸ resulting from hypertrophic or hyperplastic changes in the lining cells.¹⁹ GGT is a cell surface

Table 3 Changes in pulmonary surfactant with time after instillation with volcanic dusts and their mineral controls

Dust	Week 1	Week 3	Week 9
NaCl	0.47	0.84	0.82
Mon 1	0.88	1.10	1.00
Mon 2	0.70	0.43	1.02
Antigua	0.70	0.92	1.60
Lab	0.55	0.70	0.90
Ano	0.64	0.89	0.39
Cris/obs	0.60	0.60	0.60
Cris	1.60	2.17	1.98
DQ12	0.79	0.93	1.48

χ^2 with ties = 15.640 with 8 df; $p = 0.0478$.

Mon 1, Montserrat 1; Mon 2, Montserrat 2; Ano, anorthite; Lab, labradorite; Cris/obs, cristobalite/obsidian.

enzyme, present in alveolar epithelial type 2 and bronchiolar Clara cells,¹³ involved in the recycling of reduced glutathione. GGT activity in lavage samples has been shown to increase following damage produced by nitrogen dioxide.²⁰ Changes in pulmonary surfactant following exposure to the volcanic and control dusts are variable and cannot be subjected to statistical analysis as they are derived from pooled samples (table 3). Nevertheless, animals treated with cristobalite (all time points), DQ12 quartz (week 9), and Antigua dust (week 9) appear to have increased levels in comparison with sham treated controls. The volcanic dusts or the feldspars do not cause any significant change in GGT activity detected in the lavage samples (fig 5). By direct contrast, animals treated with both cristobalite and DQ12 quartz showed significant increases in GGT activity at the lung surface at all time points measured. Interestingly, rats instilled with the cristobalite/obsidian mixture also showed increased activities of surface GGT at one and three weeks, but this effect was not sustained to the nine week time point (fig 5).

Figure 6 shows a comparison of the size of thoracic lymph nodes removed from animals treated with the different samples for nine weeks. Only animals instilled with cristobalite or DQ12 quartz show a large increase in size compared with sham treated animals. These same effects are seen at one and three weeks post-instillation (data not shown).

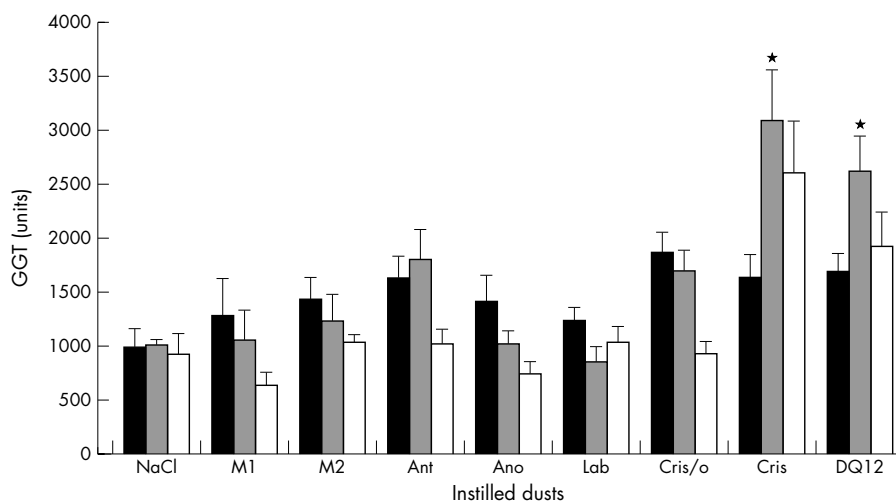


Figure 5 Changes in GGT activity in lavage fluids with time after instillation with volcanic dusts and their mineral controls (ANOVA for differences between groups: $F = 1.23$, NS; $F = 6.17$, $p < 0.0001$; $F = 6.55$, $p < 0.0001$, for weeks 1, 3, and 9 respectively). *Denotes significantly different ($p < 0.05$) from sham treated control (NaCl only). Data bars and abbreviations for dusts are as described in fig 1.

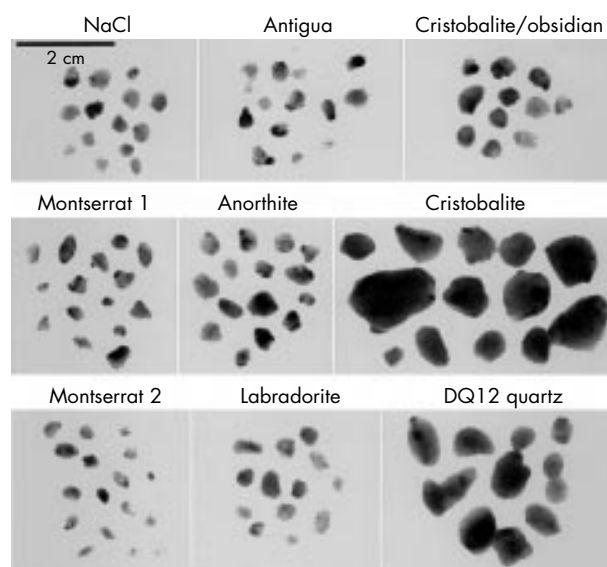


Figure 6 Bronchothoracic lymph nodes removed nine weeks post-instillation from rats treated with volcanic dusts and their mineral controls. Scale bar = 2 cm.

DISCUSSION

An assessment of the bioreactivity of respirable samples of volcanic dusts from the islands of Montserrat and Antigua was undertaken as concern has been expressed that the crystalline nature of these materials, particularly with respect to silica (cristobalite) content, may provide a catalyst for respiratory disorders in exposed individuals.^{3,4} Cristobalite is rare in nature, but may be common in high silica volcanic environments,⁹ and like crystalline quartz, exhibits a high bioreactivity.²¹

The data indicate that Montserrat respirable ash derived from pyroclastic flow (20.1% cristobalite) or phreatic explosion (8.6% cristobalite) has minimal bioreactivity in the lung. Thus, there is little or no quantitative evidence of increases in lung permeability (lung:body weight, acellular surface protein), inflammatory changes (free cell accumulation), or damage to epithelial cells (lavage increases in type 1 or 2 cell markers, pulmonary surfactant). The major component of Montserrat ash, anorthite, shows similar, low acute bioreactivity.

A number of other instillation studies with volcanic ash have been reported.²² In all these studies with rats, very high doses (10–500 mg) were instilled, detailed characterisation of the particles were not carried out, and “inert” dust controls were often omitted. Therefore, comparison with the present studies is difficult. Instillation of low levels of volcanic ash (0.3 mg/rat) were reported to be without effect on lung wet weight or hydroxyproline levels after six months of exposure.²³

In direct contrast, animals treated with a pure cristobalite (untreated or acid washed) show progressive increases in permeability and inflammation, evidence of type 2 cell hyperplasia and damage (increases in GGT), and the initiation of a mild lipoproteinosis (accumulation of surface surfactant). In addition, these extensive changes at the lung surface are mimicked by large increases in the size of thoracic lymph nodes, probably resulting from translocation of the cristobalite and/or inflammatory products during lymphatic drainage. Previous inhalation studies with rats⁷ exposed to high levels of cristobalite (39 mg/m³ for eight days), resulting in a lung deposition of 1 mg of mineral, have shown similar biological responses as noted in the present instillation studies. The DQ12 quartz produces similar effects to the pure cristobalite. The natural cristobalite (cristobalite/obsidian, 40% cristobalite) did show some reaction with the epithelium by inducing significant elevations in lavage rT1₄₀, suggesting type 1 cell damage. However, the effects of the mineral appear transient in that elevations of lavage GGT, suggesting increases in type 2 cell numbers/damage, at one and three weeks is not found at nine weeks post-instillation.

The high bioreactivity of cristobalite cannot simply be explained by the nature of its physical properties. Indeed, the mean equivalent spherical diameter of the pure cristobalite is somewhat higher than that noted in all the other samples. Thus, the pure cristobalite would have less particles/g mass to react with the lung, as the larger particles contribute the greatest proportion to mass. Bioreactivity of the cristobalite (and quartz) is therefore best explained by its reactive surface properties.²¹ The reasons why the cristobalite content, particularly of Montserrat 1 produces little or no effect, can only be surmised. Possibly these acute studies are not sufficiently long term for detrimental effects to be detected, especially if the cristobalite content is less than 40%. Alternatively, in the melt temperatures (850°C) of ash formation, the cristobalite reactive surface may be masked by layers of non-reactive volcanic glass. Similar suggestions have been made for coal dusts containing quartz.²⁴

In conclusion, two volcanic ash samples from Montserrat and a dispersed sample to the neighbouring island of Antigua have very little biological activity following short term deposition in rat lung, despite containing between 5% and 20% cristobalite. Similar, low bioreactivity is observed with anorthite, the major component of the volcanic ash.

ACKNOWLEDGEMENTS

This work was funded by the Royal Society (UK). The authors wish to thank Dr M McElroy, The Rayne Institute, University of Edinburgh, UK, for the kind gift of rT1_{40r}, and the DETR (UK) and NERC (UK) for grant support for KB and TJ, respectively.

Authors' affiliations

D G Housley, K A Bérubé, S Anderson, R J Richards, School of Biosciences, Cardiff University, PO Box 911, Museum Avenue, Cardiff CF1 3US, Wales, UK

T P Jones, Department of Earth Sciences, Cardiff University, PO Box 914, Park Place, Cardiff CF10 3YE, UK

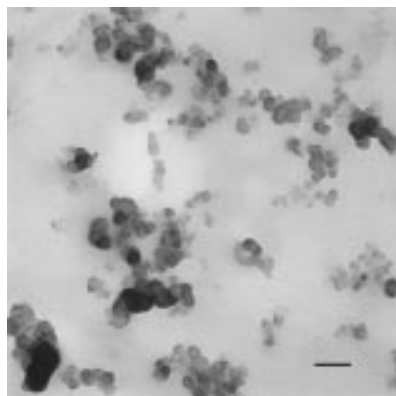
F D Pooley, School of Engineering, Cardiff University, PO Box 917, The Parade, Cardiff CF2 1XH, UK

REFERENCES

- 1 **Rea JW**. The volcanic geology and petrology of Montserrat, West Indies. *J Geol Soc London* 1974;**130**:341–66.
- 2 **Montserrat Volcano Observation Team**. The ongoing eruption in Montserrat. *Science* 1997;**276**:371–2.
- 3 **Baxter P**, Bobadonna C, Dupree R, et al. Cristobalite in volcanic ash of the Soufriere Hills volcano, Montserrat, British West Indies. *Science* 1999;**283**:1142–5.
- 4 **Nicholson R**, Hards V, Vickers B. Montserrat ash: a potential hazard to health. *Br Geol Survey* 1999;**14**:10–12.
- 5 **Murphy S**, Bérubé KA, Pooley F, et al. The response of lung epithelium to well characterised fine particles. *Life Sci* 1998;**62**:1789–99.
- 6 **Green G**, Watson A. Relation between exposure to diesel emissions and dose to the lung. In: Health Effects Institute, ed. *Diesel exhaust: a critical analysis of emissions, exposure and health effects*. Massachusetts: HEI, 1995:141–64.
- 7 **Hemenway D**, Absher, M, Trombley L, et al. Comparative clearance of quartz and cristobalite from the lung. *Am Indust Hyg Ass J* 1990;**51**:363–9.
- 8 **Horwell C**. Personal communication. Department of Earth Sciences, University of Bristol, Wills Memorial Building, Queens Road, Bristol BS8 1RJ, UK, 2000.
- 9 **Bérubé K**, Jones T, Williamson B, et al. Physicochemical characterization of diesel exhaust particles: factors for assessing biological activity. *Atmos Environ* 1999;**33**:1599–614.
- 10 **Smith D**. Opal, cristobalite and tridymite: noncrystallinity versus crystallinity, nomenclature of the silica minerals and bibliography. *Powder Diffr* 1998;**14**:2–19.
- 11 **Williamson B**, Jones T. Mineralogical and geochemical analyses. In: Jones T, Rowe NP, eds. *Fossil plants and spores: modern techniques*. London: Geological Society of London, 1999:161–5.
- 12 **Lowry O**, Rosebrough N, Farr A, et al. Protein measurement with the Folin Phenol reagent. *J Biol Chem* 1951;**193**:265–75.
- 13 **Dinsdale D**, Green A, Mason M, et al. The ultrastructural immunolocalisation of gamma-glutamyl transpeptidase in rat lung: correlation with the histochemical demonstration of enzyme activity. *Histochem J* 1992;**24**:144–52.
- 14 **McElroy M**, Pittet F, Hashimoto I, et al. A type 1 cell-specific protein is a biochemical marker of epithelial injury in a rat model of pneumonia. *Am J Physiol* 1995;**273**:L1228–34.
- 15 **StataCorp**. *Stata Statistical Software, release 7.0*. College Station, TX: Stata Corporation, 2001.
- 16 **Richards R**, Masek M, Brown R. Biochemical and cellular mechanisms of pulmonary fibrosis. *Toxicol Pathol* 1991;**19**:526–39.
- 17 **Dobbs L**, Williams C, Gonzalez R. Monoclonal antibodies specific to apical surfaces of rat alveolar type I cells bind to surfaces of cultured, but not freshly isolated, type II cells. *Biophys Biochem Acta* 1988;**970**:146–56.
- 18 **Richards R**, Curtis C. Biochemical and cellular mechanisms of dust-induced fibrosis. *Environ Health Perspect* 1984;**55**:393–416.
- 19 **Kawanami O**, Ferrans V, Crystal R. Structure of alveolar epithelial cells in patients with fibrotic lung disorders. *Lab Invest* 1982;**46**:39–53.
- 20 **Hoofman R**, Kuper C, Appelman L. Comparative sensitivity of histopathology and specific lung parameters in the detection of lung injury. *J Appl Toxicol* 1988;**8**:59–65.
- 21 **Fubini B**, Bolis V, Cavenago A, et al. Physicochemical properties of crystalline silica dusts and their possible implication in various biological responses. *Scand J Work Environ Health* 1995;**21**:9–14.
- 22 **Martin T**, Wehner A, Butler J. Evaluation of physical health effects due to volcanic hazards: the use of experimental systems to estimate the pulmonary toxicity of volcanic ash. *Am J Public Health* 1986;**76**:59–65.
- 23 **Raub J**, Hatch G, Mercer R, et al. Inhalation studies of Mt. St. Helens volcanic ash in animals. *Environ Res* 1985;**37**:72–83.
- 24 **Le Bouffant L**, Daniel H, Martin J, et al. Effect of impurities and associated minerals on quartz toxicity. *Ann Occup Hyg* 1982;**25**:625–34.

ECHO

Sizing up the evidence



Electron micrograph of diesel particles from a vehicle exhaust pipe, showing carbonaceous ultrafine particles singly and in aggregates. Bar=100nm.

Vehicle exhausts—major contributors to polluted air—are detrimental to children's health, as epidemiological and experimental evidence reviewed by Grigg in *Archives of Disease in Childhood* shows.

Diesel exhaust particles—a carbon core plus any number of adsorbed high molecular weight substances—are the primary particles in polluted air. Particulate matter (PM), comprising coarse, <10 µm (PM₁₀); fine, <2.5 µm (PM_{2.5}); and ultrafine, < 0.1 µm (UF) particles, are inhaled and the smallest penetrate to the alveoli. Vehicle exhausts and other fuel emissions produce fine and ultrafine particles, and the more technically advanced engines can generate more ultrafine particles.

According to large epidemiological studies total infant mortality is associated with exposure to PM₁₀. Short term effects on lung function are considered small in healthy children but greater with underlying respiratory disease, and these may be underestimated because of confounders and imprecise measures of individual exposure. Studies of children living by roads—affording a better indication of individual exposure—conflict owing to insufficient control of confounders and because GPs not paediatricians see most children with respiratory health problems. Experimental data show that cytokines and proinflammatory mediators are induced in lung cells by diesel exhaust particles *in vitro*, and in children the ultrafine component reaches alveolar macrophages.

A prospective birth cohort study with optimum measures of individual exposure and respiratory function would establish whether PM₁₀ causes disease as well as worsening it.

Once the size of the effects and the most toxic fraction are known, unravelling how these particles cause harm will be possible and, maybe, whether they initiate asthma.

▲ *Archives of Disease in Childhood* 2002;**86**:79–83.



Please visit the Occupational and Environmental Medicine website [www.occenvmed.com] for link to this full article.