

Original Article

Persulphate challenge in female hairdressers with nasal hyperreactivity suggests immune cell, but no IgE reaction

Kerstin Kronholm Diab¹ , Lennart Truedsson², Maria Albin¹ and Jørn Nielsen¹

(1) Department of Occupational and Environmental Medicine, University Hospital, 221 85 Lund, Sweden

(2) Department of Clinical Microbiology and Immunology, University Hospital, 221 85 Lund, Sweden

 Kerstin Kronholm Diab
Email: kerstin.diab@med.lu.se

Received: 16 April 2008 Accepted: 13 November 2008 Published online:
2 December 2008

Abstract

Purpose The aim of this study was to examine the effects of persulphate on the nasal mucosa and on the immune cells in hairdressers suffering from bleaching powder associated rhinitis (BAR) versus subjects with rhinitis not previously exposed to bleaching powder.

Methods Fifteen hairdressers (S) with BAR, 14 without symptoms (WS) and 12 atopics (A) with rhinitis but without exposure to bleaching powder were studied. Each performed a nasal challenge with persulphates. Effect parameters were symptom score, acoustic rhinometry, albumin in nasal lavage, subpopulations of lymphocytes in blood and specific serum antibodies.

Results The S group had a post-challenge increase in nasal symptoms and nasal lavage albumin. The A group reacted to a lesser extent. The S and A groups showed an increase in Th1 cells. An HLA class II cell expression was noticed in both groups of hairdressers.

No evidence of a type 1 reaction (immediate type) to persulphate was noticed.

Conclusions Persulphate challenge affects hairdressers with BAR, but also atopics. The reaction may be driven by a Th1 cell activation.

Keywords Bleaching powder - Hairdressers - IgE - Nasal challenge - Persulphate - Rhinitis

Introduction

Hairdressers are frequently exposed to several potential agents which are irritating to the airways and promote immune-mediated reactions. Epidemiological studies have shown an increased risk for work-related airway symptoms in hairdressers (Leino et al. [1997](#), [1998](#); Albin et al. [2002](#); Brisman et al. [2003](#)). Albin et al. ([2002](#)) showed that among the hairdressers who could point out a causal agent for the symptoms from the airways, more than 80% named the bleaching powder.

Bleaching powder contains persulphates, agents earlier shown to be associated with occupational asthma although the mechanism has not elucidated (Parra et al. [1992](#); Macchioni et al. [1999](#); M•noz et al. [2003](#); Moscato et al. [2005](#)). However, in hairdressers, rhinitis is more frequent than asthma (Albin et al. [2002](#); Brisman et al. [2003](#)). The association between persulphate exposure and rhinitis has not been extensively studied. The mechanism for development of bleaching powder associated rhinitis is still unclear and relevant diagnostic methods are lacking. These challenges make vocational advising of symptomatic hairdressers difficult.

The aim of this study was to examine the effects of persulphate on the nasal mucosa and on the immune cells in hairdressers who primarily suffer from bleaching powder associated rhinitis versus subjects with rhinitis who has not been previously exposed to bleaching powder.

Material and methods

Subjects

Three groups were studied. The target group consisted of female hairdressers with nasal symptoms mainly related to bleaching powder (S, $n = 15$). Two comparison groups were employed, one of female hairdressers (WS, $n = 14$) without symptoms, another of atopic

(by history) females without professional contact to bleaching powder (A, $n = 12$). None of the hairdressers had a history of atopy. At the time of the challenge, no subjects were taking allergy medications. Participants in the S group were chosen as those from a group of 33 hairdressers complaining of nasal symptoms related to bleaching powder. The fifteen S group participants were deemed to have the worst symptoms associated with bleaching powder. The inclusion and exclusion criteria can be seen in Table 1. The study was approved by the local ethical committee. Study participants gave written informed consent.

Table 1 Criteria of the participating groups, symptomatic (S) and asymptomatic hairdressers (WS) and atopics (A)

	S group ($n = 15$)	WS group ($n = 14$)	A group ($n = 12$)
Inclusion criteria	Only work-related nasal symptoms from bleaching powder	No work-related airway symptoms	No exposure to bleaching powder Nasal symptoms Atopy by history
Exclusion criteria	Asthma Atopy by history	Asthma Atopy by history	Asthma

The mean age in each group was in the S group 39 (SD 8), in the WS group 38 (SD 12), and in the A group 39 (SD 12) years. The hairdressers had been employed as such for 17 years (mean, SD 6 in the S and 12 in the WS group). The exposure time until appearance of symptoms in the S group was 8 years (mean, SD 5). Four subjects in the S group, two in the WS and all but two in the A group showed a positive standard skin prick test. There were no smokers in group A, three in group S and four in group WS. In the S group, 12 subjects had nasal blockage, 11 had a runny nose, and 12 sneezing. The corresponding figures for group A were 12, 10 and 9. Although the inclusion criteria was that the hairdressers should not have any known allergies, four hairdressers in the S and two in the WS groups showed a positive reaction to common allergens. Two participants in the atopic group had no positive reaction in spite of having allergy symptoms.

Medical examination before challenge

A standardized interview was conducted with a physician. This included a medical and occupational history, questions about atopy and smoking habits. Airway related symptoms and their relationship to the workplace were thoroughly discussed. Work-related symptoms were defined as those worsened at the workplace and/or recovery during weekends or holidays away from the workplace. Atopy by history was defined as having a history of hay fever, asthma or atopic eczema in childhood or adolescence. A physical examination was performed including rhinoscopy to exclude any nasal condition that may mimic or generate rhinitis-like symptoms.

Skin prick test with 13 common allergens (ALK, Copenhagen, Denmark) was performed. Furthermore, the hairdressers were tested stepwise with fresh solutions [0.05, 0.1 and 0.5% (w/v)] of potassium persulphate in sterile water. The reaction was read according to Aas and Belin ([1973](#)). Positive reactions were scored when the area of the wheal was at least half of that induced by a histamine solution (10 mg/mL).

Specific nasal challenge

The nasal challenge was performed with a 0.001% fresh solution of potassium persulphate in isotonic saline solution, and after 20 min with a 0.01% solution (w/v) using a De Vilbiss spray (atomizer No. 15) as earlier described (Andersson et al. [1987](#)). A total of 300 •g of each solution was sprayed into the nasal cavities by turns. The spraying was performed immediately after a maximal inspiration to prevent the solution from entering the lower airways (Melillo et al. [1997](#)).

Nasal symptoms were recorded using a score system according to Malm et al. ([1981](#)) before, 15 min after each challenge, 2 and 5 h after the last challenge. Nasal blockage and secretions were each rated on a scale 0–3 (no symptoms = 0; severe symptoms = 3). The rating was performed for each nose cavity and then the average was used. The number of sneezes was counted and scored (no sneeze attacks = 0; 1–5 = 1; 6–15 = 2; >15 = 3). A combined nasal symptom score was calculated by addition of the score for each symptom.

When the reaction of individual subjects were studied, a significant response was defined as a subject increasing in total symptom score more than three standard deviations from the mean change in the WS group.

Acoustic rhinometry (AR) was performed using a RHIN 2000 (RhinoMetrics A/S, Lyngø, Denmark) according to Hilberg and Pedersen ([2000](#)). The anatomical nosepieces were chosen according to the shape of the subject's nostril. The probe was handheld; the subject sat in an upright position with the neck fixed breathing quietly through the mouth. During the measurement the subjects held their breath. The room was quiet and the room temperature was standardized. The Minimum Crosssectional Area (MCA) in cm² was

measured in the distance of 10–32 mm from the nares before the first challenge, 15 min after this and 15 min, 2 h and 5 h after the second challenge. Three consecutive approved measurements for each nostril were performed and the mean value was registered for each side of the nose. Data on nasal dimensions are presented as the mean value of the values for the right and the left side of the nose.

A nasal lavage was performed twice before the first challenge and 20 min, 2 h and 5 h after the second challenge directly after the rhinometric measurement. The first lavage was performed to wash out mediators developed by processes before the challenge. The second lavage before the challenge was used as the baseline for the post-challenge samples. The lavage was performed with a syringe attached to a nasal olive (Nasaline; ENTpro AB, Stockholm, Sweden). Fifteen milliliters of sterile isotonic saline solution at 35°C was introduced into the nasal cavity with the patient sitting in a forward flexed neck position. The transmission was stopped when solution appeared in the opposite nostril. The solution was sucked backwards into the syringe and injected again. This procedure was repeated three times in the left and the right nostril alternately. The samples were immediately cooled and frozen at –70°C.

Analysis of nasal lavage and blood tests

The levels of albumin, eosinophil cationic protein (ECP) and myeloperoxidase (MPO) in the supernatants of the nasal lavage fluids were analyzed by double antibody radioimmunoassay. The level of tryptase was analyzed by a double antibody fluoroenzyme immunoassay. These assays are available as commercial kits (Pharmacia Diagnostics AB, Uppsala, Sweden). The detection limit for albumin was 0.4 mg/L, for ECP 2.0 •g/L, for MPO 2.0 •g/L and for tryptase 1.0 •g/L.

Venous blood was collected before the challenge and 5 h after the last challenge. One tube without additives was centrifuged and the serum was frozen at –20°C for analysis of specific antibodies of IgE class. Samples were also taken in tubes with heparin and with EDTA, respectively, for blood cell analysis within 6 h after blood sampling.

Analysis for specific IgE antibodies against persulphates was performed by specific immunoblotting (Johannesson et al. [2001](#)). Aliquots of the conjugate with persulphate and proteins from nasal lavage fluid (NLF) were separated by 4–12% SDS-PAGE. The proteins were blotted to a preconditioned PVDF (polyvinylidene difluoride) membrane (Biorad, Hercules, CA, USA) using a Semidry Electrobloetter A (Ancos, København, Denmark) at 150 mA for 3 h. The membrane was then blocked by incubation in 5% dried milk. Sera from the hairdressers were used as primary antibodies. The secondary antibody was I¹²⁵ IgE. The membranes were wrapped in cellophane and exposed on CEA RP X-ray film (CEA group, Strängnäs, Sweden) for 1 month.

Flow cytometry was used to measure the concentration of leukocytes, lymphocytes and lymphocyte subpopulations. Leukocyte populations were distinguished by their light-scattering abilities; forward scatter reflects the cell size and side scatter reflects the complexity/granularity. The different lymphocyte populations distinguished were T cells (CD3+), T helper (Th) cells (CD3+, CD4+), T cytotoxic cells (CD3+, CD8+), B cells (CD19+) and NK cells (CD3–, CD16+or CD56+). Also HLA-DR expression on lymphocytes and on the T cell subpopulation was determined (Syk et al. [1999](#)). In short, samples of 5 •L of fluorescence-labeled monoclonal antibodies were added to 50 •L of blood and incubated for at least 10 min. The erythrocytes were then lysed by Immunoprep (Coulter, Miami, FL, USA), and the sample analyzed on a flow cytometer (Coulter Epics II, Coulter Electronics, Hialeah, FL, USA).

Analysis of Th1 and Th2 lymphocytes was done by measurement of the intracellular expression of the cytokines interferon- γ and IL-4 using flow cytometry (Jung et al. [1993](#); Picker et al. [1995](#)). A minimum of 3,000 cells was analyzed within the lymphocyte cluster, and the results are expressed as a percentage of cytokine-producing cells in a population of specified T helper cells, i.e., CD3 and CD4 positive cells (Källström et al. [2002](#)).

Statistical analysis

Wilcoxon's signed rank test was used to compare paired data, whereas Mann–Whitney's *U* test was used in tests between the groups. Stratification was used to adjust for possible confounding factors. *P* value of <0.05 was considered significant (two-tailed). Data were analyzed by SPSS for Windows 12.0 (SPSS Inc., Chicago, IL, USA). Stratification was done using StatXact 6 (Cytel Software Corporation, Cambridge, USA).

Results

In skin prick testing of the hairdressers, there was no reaction to persulphates. Before the challenge, the S group had a higher total score than the other groups. The A group had the next highest and both S and A differed from the WS group (Table [2](#)). The S group had a significant increase in the total symptom score during and 2 h after the last challenge. The A group symptom score increased only during the challenge. No increase was noted within the WS. The smokers and the skin prick test positive subjects did not differ from the rest of their groups, respectively, (data not shown). When individual subjects were studied, six subjects in the S and two in the A group reacted significantly to the challenge.

Table 2 Total symptoms score (the combined score from nasal blockage, secretion and number of sneezes; median), before, during and 2 and 5 h after nasal challenge with persulphates in symptomatic (S) and asymptomatic (WS) hairdressers and in atotics (A)

	S group (n = 15)	WS group (n = 14)	A group (n = 12)
Baseline	2.0***	0	0.5*
15 min after challenge 1	4.0 ^{aa}	0	1.5 ^a
15 min after challenge 2	5.0 ^{aaa}	0	2.5 ^{aa}
2 h after challenge 2	3.0 ^a	0	0.5
5 h after challenge 2	2.0	0	1.5

Significant differences between WS group and S and A groups, respectively, * $P \leq 0.05$, *** $P \leq 0.001$

Significant differences within the groups compared to the baseline ^a $P \leq 0.05$ ^{aa} $P \leq 0.01$ ^{aaa} $P \leq 0.001$

The results of the acoustic rhinometry showed a decrease in MCA of 7% after the first and 14% after the second challenge in the S group compared to the baseline level (0.96; 0.58–1.65 vs. 0.83; 0.53–1.41 cm²; median and range). In the A group, a transient decrease of 14% was noticed after the first challenge, but the group returned to baseline after the second one. Otherwise there were no changes in the groups compared to the baseline levels.

Albumin in the first lavage fluid was raised in both the S and the A groups compared to the WS group (Table 3). The differences were not statistically significant when adjusted for smoking. At the second pre-challenge lavage, all three groups showed a decrease in albumin levels. Twenty minutes after the second challenge, albumin levels transiently but significantly increased in the S group (Table 3). In the two groups of hairdressers, there was no difference amongst the current smokers and the skin prick test positive subjects.

Table 3 Albumin (median; range) in nasal lavage fluid

Albumin, mg/L	S group (n = 15)	WS group (n = 14)	A group (n = 12)
Time 0	5.5 (0.4–21.6)	3.4* (0.6–20.2)	13.0 (0.7–39.7)
Baseline	1.9 ^b (0.2–17.0)	1.2 (0.05–4.0)	3.1 (0.7–17.7)
20 min after challenge 2	4.1 ^a (0.0–21.2)	1.5 (0.1–9.3)	2.6 (0.4–14.1)
2 h after challenge 2	2.7 (0.0–35.9)	1.8 ^a (0.3–36.2)	1.9 (0.7–15.9)
5 h after challenge 2	4.8 (0.3–51.1)	2.2 (0.4–45.6)	4.4 (0.6–21.6)

Groups and nasal challenge like in Table [2](#)

Significant differences between the groups WS and A, * $P \leq 0.05$

Significant differences within the groups compared to the baseline, ^a $P \leq 0.05$

^b14 persons

No statistically significant differences in ECP levels appeared between the groups or in relation to the challenge (Table [3](#)). For MPO, no significant differences were found between the groups at time 0. After the first lavage, a decrease was observed in all three groups. Thereafter a gradual increase was noticed during the observation time. No differences were noticed between the groups (data not shown). None of the subjects had elevations of tryptase levels above the detection level.

No significant differences between the groups were found for any of the cell populations studied (Tables [4](#), [5](#), [6](#)). In all three groups, the concentrations of lymphocytes and leukocytes in blood increased significantly from before to after the challenge (Table [4](#)). The result suggests that the increase in lymphocytes was more prominent in group S and A.

Table 4 Concentrations of leukocytes and lymphocytes in peripheral blood (median; range) pre- and 5 h post-challenges with persulphates. Groups like in Table [2](#)

	S group (n = 15)	WS group (n = 14)	A group (n = 12)
Leukocytes × 10 ⁹ /L			
Pre-challenge	7.0 (3.4–11.4)	5.8 (4.7–6.9)	6.7 (3.4–8.2)
5-h post-challenge	8.2 ^a (3.7–12.9)	6.9 ^{aa} (5.1–9.2)	7.2 ^{aa} (4.6–8.8)
Lymphocytes × 10 ⁹ /L			
Pre-challenge	2.6 (1.3–3.7)	2.4 (1.3–4.8)	2.0 (1.1–3.8)
5-h post-challenge	3.3 ^{aa} (1.4–4.3)	2.5 ^a (1.2–3.9)	2.6 ^a (1.5–3.5)

Significant differences within the groups from pre- to post-challenges ^a $P \leq 0.05$ ^{aa} $P \leq 0.01$

Table 5 Portion of Th1 and Th2 lymphocytes and HLA-DR expression on T lymphocytes (median; range) pre- and 5-h post-challenges with persulphates

Blood	S group (n = 15)	WS group (n = 14)	A group (n = 12)
Th1 in % of CD4 ⁺ cells			
Pre-challenge	17.0 (9.9–30.0)	15.5 (7.8–29.0)	15.5 (11.0–43.0)
5-h post-challenge	19.0 ^a (11.0–36.0)	15.0 (11.0–30.0)	19.0 (11.0–38.0)
Th2 in % of CD4 ⁺ cells			
Pre-challenge	2.9 (0.8–4.7)	2.4 (1.3–4.8)	2.0 (1.2–9.4)

5-h post-challenge	3.0 (0.8–4.8)	2.5 (0.9–4.5)	2.1 (1.1–8.1)
HLA-DR T cells × 10 ⁹ /L			
Pre-challenge	0.11 (0.04–0.71)	0.10 (0.04–0.28)	0.11 (0.03–0.46)
5-h post-challenge	0.12 ^a (0.04–0.81)	0.12 ^a (0.02–0.43)	0.10 (0.04–0.61)

Groups like in Table 2

Significant differences within the groups from pre- to post-challenges, ^a $P \leq 0.05$

Table 6 Distributions of lymphocyte subpopulations in peripheral blood pre- and 5-h post-challenges with persulphates

Blood	S group (n = 15)	WS group (n = 14)	A group (n = 12)
CD 3 T cells %			
Pre-challenge	75.0 (67.0–87.0)	75.5 (66.0–86.0)	74.5 (58.0–90.0)
5-h post-challenge	75.0 (68.0–86.0)	74.0 (66.0–87.0)	74.5 (63.0–86.0)
CD 3.4 T helper cells %			
Pre-challenge	48.0 (37.0–58.0)	45.0 (34.0–56.0)	47.0 (37.0–66.0)
5-h post-challenge	48.0 (41.0–59.0)	45.0 (38.0–61.0)	50.0 (37.0–62.0)
CD 3.8 T Cytotox %			
Pre-challenge	28.0 (15.0–34.0)	27.5 (9.1–40.0)	23.5 (12.0–37.0)

5 h post-challenge	25.0 ^{aa} (12.0–36.0)	25.0 ^{aa} (7.8–35.0)	23.0 ^{aa} (9.1–36.0)
CD16 + CD56 NK cells%			
Pre-challenge	11.0 (2.8–19.0)	9.8 (3.4–26.0)	8.5 (4.4–23.0)
5 h post-challenge	11.0 (1.0–16.0)	8.8 (4.2–21.0)	12.5 (6.7–18.0)
CD 19 B cells %			
Pre-challenge	10.0 (3.0–15.0)	8.1 (4.7–17.0)	8.1 (0.6–16.0)
5-h post-challenge	9.0 ^a (4.3–18.0)	7.7 (5.5–19.0)	8.5 (0.9–16.0)

Groups like in Table [2](#)

Significant differences within the groups pre- and post-challenges, ^a $P \leq 0.05$ ^{aa} $P \leq 0.01$

The Th1 cells increased after challenge in the S group. The Th2 cells did not increase. The same tendency was observed in the A group although this was not significant. No changes were seen in the WS group (Table [5](#)). With regard to HLA class II expression (DR) on T cells after challenge, there was a significant increase in both the S and the WS group. Thus, there was an increase in HLA-DR expression in T cells, which reflects activation in both groups of hairdressers, but not in the atopics.

The portion of T helper cells in the circulation remained unchanged after challenge indicating that the increase in lymphocyte concentration was dependent mainly on expansion of the T helper cell population (Table [6](#)). However, the concentration of T cytotoxic cells was unchanged; therefore, the percentage of T cytotoxic cells was decreased after challenge. These changes were seen in all three groups. A small increase in B cell concentration was also seen, although the portion of B cells falls a little due to the increase in T cells. This alteration in cell concentration was significant for both the S and A group. There were no changes in numbers of NK cells (Table [6](#)).

No IgE antibodies were detected at immunoblotting using persulphate modified nasal proteins as allergens in the sera in any of the groups.

Discussion

To our knowledge, this is the first study that compares the effects of nasal challenge with persulphate in hairdressers with symptoms predominantly associated to bleaching powder to atopsics without exposure to persulphates. Nasal symptoms increased in both the symptomatic hairdressers and the atopsics, but not in the asymptomatic group. The S group developed the most symptoms and a rise in albumin in the nasal lavage liquid, which was not found in the A group. In spite of a low challenge dose, persulphates had an impact on the mobilization of specific types of immune cells in the blood circulation, such as Th1 cells in the S group, but the change only marginally differed from that of the A group. No subjects reacted to the skin prick test with the persulphate solution. The immune blotting study showed no signs of specific antibodies. Thus, this study does not demonstrate an IgE mediated allergic reaction to persulphate in the symptomatic hairdressers.

Because the A group was not earlier exposed to bleaching powder, the reaction in this group is probably not specific. A positive reaction to persulphate in a non-persulphate exposed asthmatic was also reported by M•noz et al. ([2004](#)). The stronger reaction of the S group may indicate a reaction to persulphate that is different from that of the atopsics. However, it is more likely that the hairdressers who had more symptoms before the challenge had a less specific reaction to persulphates than group A. This point of view supports the results from an earlier study (Hytönen et al. [1997](#)), in which specific reactions to persulphates were rare. Thus, we think that at least most of the reactions among the hairdressers also may be of a not specific nature.

In this study, we only challenged subjects with potassium persulphate. Hairdressers are often exposed to other persulphates as well. Thus, reactions to those types of persulphates may have been missed. However, immunologic differences between the used persulphates have not been indicated (Pang and Fiume [2001](#)). M•noz et al. ([2004](#)) showed that persulphate sensitive asthmatics reacted in skin prick test equally to potassium and ammonium persulphates.

In spite of the clear association between exposure to bleaching powder and symptoms in the medical history, the hairdressers who did not react significantly in the challenge test may react to agents other than persulphates in the hairdresser environment. Thus, Hollund et al. ([2002](#)) found that more than 5% of the hairdressers in a cross sectional study had allergy to latex. The same figure was mentioned by Moscato et al. ([2005](#)), even if in this later study persulphate was by far the most frequent causative agent.

None of the participants in the study reacted in the skin prick test to persulphate. In our

clinic, we infrequently find positive tests in hairdressers with bleaching powder associated asthma. In the study of Moscato et al. (2005) no positive skin prick test was found, whereas half of the patients with asthma in the study of M•noz et al. (2004) were positive. A positive skin prick test may not necessarily mean demonstrable specific antibodies. Aalto-Korte and Mäkinen-Kiljunen (2003) found seven out of 138 patients tested with persulphates to have positive skin prick tests. Only one of five tested sera had demonstrable antibodies. Thus, the mechanism of persulphate associated reactions is not clear even if a positive skin prick test has been recorded.

All three groups had an increase in white blood cells post-challenge, which may be explained by something else other than that of the challenge. However, some differences in lymphocytes subpopulations were noticed. The S group had an increase of T helper cells, particularly Th1 cells. In addition, an increase in activated T cells assessed by HLA class II expression was observed. The changes only marginally differed from those of the A group, whereas the changes in the WS group were minimal. This indicates an effect of the challenge in the two symptomatic groups in spite of the low challenge dose.

An association has earlier been described between levels of Th1 cells and exposure to other reactive low molecular agents in animals (isocyanates and ozone, van Loveren et al. 1996). It was hypothesized that Th1 cells after exposure to these agents may play a role in induction of asthmatic disorders of non IgE origin. Thus, the increase in Th1 cells as noticed in our study may be a part of the process of hyperreactivity. It is well known that high exposure to irritants can cause bronchial as well as nasal hyperreactivity (Rom 1992; Leroyer et al. 1999; Banauch et al. 2005) although the exact mechanism is not clear. However, almost nothing is known about chronic low irritant exposure and the development of hyperreactivity. There is a need for such studies.

In conclusion, we showed that challenge with persulphates can elicit symptoms in hairdressers with bleaching powder associated rhinitis, but also to some degree in atopics without exposure to bleaching powder. No signs of involvement of specific IgE antibodies against persulphate were found. An increase in Th1cells was noticed in both groups indicating a reaction driven by a Th1 cell activation. However, further studies are necessary to understand the mechanism for bleaching powder associated symptoms in hairdressers.

Acknowledgments We thank I. Bensryd RN, P. Aprea BT and E. Assarsson RN for assistance with the challenges; B. Björk BT, G. Johannesson Med. Lic., K. Paulsson BT, A. Andreasson BT and S. Jönsson BT for laboratory analysis and Anna Axmon, associate professor, for statistical assistance. Financial support was obtained from the Swedish Council for Working Life and Social Research (No. 2001-0217).

References

Aalto-Korte K, Mäkinen-Kiljunen S (2003) Specific immunoglobulin E in patients with immediate persulfate hypersensitivity. *Contact Dermatitis* 49:22–25. doi:[10.1111/j.0105-1873.2003.00134.x](https://doi.org/10.1111/j.0105-1873.2003.00134.x)



Aas K, Belin L (1973) Standardization of diagnostic work in allergy. *Int Arch Allergy* 45:57–60



Albin M, Rylander L, Mikoczy Z, Lillienberg L, Dahlman Höglund A, Brisman J et al (2002) The incidence of asthma in female Swedish hairdressers. *Occup Environ Med* 59:119–123.

doi:[10.1136/oem.59.2.119](https://doi.org/10.1136/oem.59.2.119)



Andersson M, Kogerer BV, Andersson P, Pipkorn U (1987) Allergen-induced nasal hyperreactivity appears unrelated to the size of the nasal and dermal immediate allergic reaction. *Allergy* 42:631–637. doi:[10.1111/j.1398-9995.1987.tb00395.x](https://doi.org/10.1111/j.1398-9995.1987.tb00395.x)



Banauch GI, Dhala A, Prezant DJ (2005) Pulmonary disease in rescue workers at the World Trade Center site. *Curr Opin Pulm Med* 11(2):160–168. doi:[10.1097/01.mcp.0000151716.96241.0a](https://doi.org/10.1097/01.mcp.0000151716.96241.0a)



Brisman J, Albin M, Rylander L, Mikoczy Z, Lillienberg L, Höglund AD et al (2003) The incidence of respiratory symptoms in female Swedish hairdressers. *Am J Ind Med* 44(6):673–678. doi:[10.1002/ajim.10293](https://doi.org/10.1002/ajim.10293)



Hilberg O, Pedersen OF (2000) Acoustic rhinometry: recommendations for technical specifications and standard operating procedures. *Rhinol Suppl* 16:3–17



Hollund BE, Moen BE, Egeland GM, Florvaag E (2002) Occupational exposure to hairdressing chemicals and immunoglobulin E synthesis. *Scand J Work Environ Health* 28:264–269



Hytönen M, Leino T, Sala E, Kanerva L, Tupasela O, Malmberg H (1997) Nasal provocation test in the diagnosis of hairdressers' occupational rhinitis. *Acta Otolaryngol Suppl* 529:133–136.

doi:[10.3109/00016489709124104](https://doi.org/10.3109/00016489709124104)



Johannesson G, Rosqvist S, Lindh CH, Welinder H, Jönsson BAG (2001) Serum albumins are the major site for in vivo formation of hapten-carrier protein adducts in plasma from humans and guinea pigs exposed to Type-1 allergy inducing hexahydrophthalic anhydride. Clin Exp Allergy 31(7):1021–1030. doi:[10.1046/j.1365-2222.2001.01109.x](https://doi.org/10.1046/j.1365-2222.2001.01109.x)



Jung T, Schauer U, Heusser C, Neumann C, Rieger C (1993) Detection of intracellular cytokines by flow cytometry. J Immunol Methods 159:197–207. doi:[10.1016/0022-1759\(93\)90158-4](https://doi.org/10.1016/0022-1759(93)90158-4)



Källström E, Roscher I, Andreasson A, Bäck O, van Hage-Hamsten M (2002) Decreased frequency of intracellular IFN- γ producing T cells in whole blood preparations from patients with atopic dermatitis. Exp Dermatol 11:556–563. doi:[10.1034/j.1600-0625.2002.110608.x](https://doi.org/10.1034/j.1600-0625.2002.110608.x)



Leino T, Tammilehto L, Luukkonen R, Nordman H (1997) Self reported respiratory symptoms and diseases among hairdressers. Occup Environ Med 54(7):452–455



Leino T, Tammilehto L, Hytonen M, Sala E, Pakkulinainen H, Kanerva L (1998) Occupational skin and respiratory diseases among hairdressers. Scand J Work Environ Health 24(5):398–406



Leroyer C, Malo JL, Girard D, Dufour JG, Gautrin D (1999) Chronic rhinitis in workers at risk of reactive airways dysfunction syndrome due to exposure to chlorine. Occup Environ Med 56:334–338



Macchioni P, Kotopulos C, Talini D, De Santis M, Masino E, Paggiaro PL (1999) Asthma in hairdressers: a report of 5 cases. Med Lav 90:776–785



Malm L, Wihl JÅ, Lamm CJ, Lindqvist N (1981) Reduction of metacholine-induced nasal secretion by treatment with a new topical steroid in perennial non-allergic rhinitis. Allergy 36:209–214. doi:[10.1111/j.1398-9995.1981.tb01836.x](https://doi.org/10.1111/j.1398-9995.1981.tb01836.x)



Melillo G, Bonini S, Cocco G, Davies RJ, de Monchy JG, Frolund L et al (1997) EAACI provocation tests with allergens. Report prepared by the European Academy of Allergology and Clinical Immunology Subcommittee on provocation tests with allergens. Allergy 52(Suppl 35):1–35. doi:[10.1111/j.1398-9995.1997.tb02538.x](https://doi.org/10.1111/j.1398-9995.1997.tb02538.x)



Moscato G, Pignatti P, Yacoub MR, Romano C, Spezia S, Perfetti L (2005) Occupational asthma and occupational rhinitis in hairdressers. *Chest* 128:3590–3598. doi:[10.1378/chest.128.5.3590](https://doi.org/10.1378/chest.128.5.3590)



M•noz X, Cruz MJ, Orriols R, Bravo C, Espuga M, Morell F (2003) Occupational asthma due to persulfate salts. *Chest* 123:2124–2129. doi:[10.1378/chest.123.6.2124](https://doi.org/10.1378/chest.123.6.2124)



M•noz X, Cruz MJ, Orriols R, Torres F, Espuga M, Morell F (2004) Validation of specific inhalation challenge for diagnosis of occupational asthma due to persulphate salts. *Occup Environ Med* 61:861–866. doi:[10.1136/oem.2004.013177](https://doi.org/10.1136/oem.2004.013177)



Pang S, Fiume MZ (2001) Final report of the safety assessment of ammonium, potassium and sodium persulfate. *Int J Toxicol* 20(Suppl 3):7–21. doi:[10.1080/10915810152630710](https://doi.org/10.1080/10915810152630710)



Parra FM, Igea JM, Quirce S, Ferrando MC, Martin JA, Losada E (1992) Occupational asthma in a hairdresser caused by persulphate salts. *Allergy* 47:656–660. doi:[10.1111/j.1398-9995.1992.tb02391.x](https://doi.org/10.1111/j.1398-9995.1992.tb02391.x)



Picker LJ, Singh MK, Zdraveski Z, Treer JR, Waldrop SL, Bergstresser PR et al (1995) Direct demonstration of cytokine synthesis heterogeneity among human memory/effector T cells by flow cytometry. *Blood* 86:1408–1419



Rom WN (1992) *Environmental and occupational medicine*, 2nd edn. Little, Brown and Company, Boston

Syk I, Andreasson A, Truedsson L, Brunkwall J, Risberg B (1999) Postoperative downregulation of MHC class II antigen on monocytes does not differ between open and endovascular repair of aortic aneurysms. *Eur J Surg* 165:1035–1042. doi:[10.1080/110241599750007856](https://doi.org/10.1080/110241599750007856)



van Loveren H, Steerenberg AP, Garssen J, van Bree L (1996) Interaction of environmental chemicals with respiratory sensitization. *Toxicol Lett* 86:163–167. doi:[10.1016/0378-4274\(96\)03686-7](https://doi.org/10.1016/0378-4274(96)03686-7)

