

Prevention of latex sensitization in guinea pigs by a bacterial and viral filter used in anaesthesia

J. Barbara^{1,2}, M.-C. Santais², D. A. Levy¹, F. Ruff^{2†} and F. Leynadier^{1*}

¹Laboratoire Universitaire de Recherche en Immuno-Allergologie (LURIA), Centre d'Allergologie, Hôpital Tenon (AP-HP), 4 rue de la Chine, F-75970 Paris Cedex 20, France.

²Laboratoire de Physiopathologie Respiratoire, Université Paris V, UFR Biomédicale, 45 rue des Saints-Pères, F-75270 Paris Cedex 06, France

*Corresponding author. E-mail: francisque.leynadier@tnn.ap-hop-paris.fr

Background. Preventing anaphylactic reactions as a result of natural rubber latex (NRL) proteins is an important concern in anaesthesia. The clinical relevance of a bacterial/viral filter (PallTM BB25) in preventing sensitization to NRL by inhalation was tested in guinea pigs.

Methods. Guinea pigs ($n=8-10$ in each group) were exposed to aerosolized NRL-contaminated cornstarch powder or to NRL in saline for 1 h every day for 2 weeks. The experiments were repeated with a PallTM BB25 filter placed over the aerosol system. Control groups were exposed to non-contaminated cornstarch or to saline alone. Three weeks after the last exposure, specific bronchial challenge was performed and thromboxane (Tx) B₂ levels in bronchoalveolar lavage fluid were measured.

Results. After bronchial challenge, the animals exposed to NRL or NRL-contaminated cornstarch with the BB25 filter in place showed a level of bronchoconstriction (i.e. the variation of pulmonary insufflation pressure) not different from controls. Conversely, those exposed to NRL or NRL-contaminated cornstarch without the filter showed a higher level of bronchoconstriction (respectively, $P<0.02$ and $P<0.001$) than control. Elevated Tx_{B2} levels were found in the lungs of the guinea pigs, which inhaled NRL or NRL-contaminated cornstarch in the absence of a filter. Animals treated with the filter showed comparable Tx_{B2} levels with those of control.

Conclusion. The PallTM BB25 filter efficiently protected the guinea pigs from sensitization to NRL. This filter can be used as a complementary measure for avoidance of NRL contact during surgical procedures particularly if the mechanical ventilator apparatus contain NRL devices.

Br J Anaesth 2005; **95**: 349–54

Keywords: airway; complications, latex allergy; equipment, filters; model, guinea pigs

Accepted for publication: April 29, 2005

Cornstarch powder is widely used in medical gloves. Cornstarch particles, instead of talc, are very volatile and are able to adsorb high quantities of natural rubber latex (NRL) proteins including those that are responsible for hypersensitivity reactions to latex.¹ Cornstarch particles bearing NRL proteins are now considered as potent allergens as it can induce respiratory disorders like common aeroallergens, such as pollens or dust mites. A correlation between the increasing use of powdered gloves in workplaces and the prevalence of latex allergy has been demonstrated.^{2,3} The use of powder-containing gloves in operating theatres induces contamination of the environment by cornstarch particles bearing NRL proteins.⁴ Furthermore, Kujala and colleagues⁵ have reported that airborne latex allergens correlate better with airborne glove powder than with the glove

allergen content. The incidence of anaphylactic reactions during anaesthesia has been estimated to 1:13 000, and NRL was responsible in 16.6% of these cases.⁶ Ruëff and colleagues⁷ have reported a correlation between the occurrence of multiple surgical procedures and the increase in prevalence of sensitization to NRL in patients. Regarding these findings, patients need relevant protection during surgical procedures. In previous studies, we developed experimental models of NRL sensitization in guinea pigs either via peritoneal or respiratory route, using cornstarch as an allergen carrier. NRL-sensitized animals demonstrated airway hyper-responsiveness induced by NRL after specific bronchial challenge. In addition, the exposure either by

[†]Deceased.

peritoneal or respiratory routes to glove powder contaminated by NRL proteins, aggravated the respiratory disorders induced by NRL.^{8,9}

We reported recently a bacterial/viral filter used in anaesthesia (Pall™ BB25) that efficiently retained the NRL proteins.¹⁰ In the present study, we tested the relevance of the Pall™ BB25 filter in prevention of NRL sensitization in guinea pigs. For this purpose the animals were placed in similar conditions of airway exposure as a patient connected to an artificial breathing system during surgical procedures. A BB25 filter was inserted at the top of the NRL-nebulizing system in order to retain NRL-contaminated cornstarch particles or NRL solubilized in saline solution. In addition, a model of sensitization to ovalbumin was also used for comparison to a well-known antigen.

Materials and methods

Animals

Male albino Dunkin–Hartley guinea pigs (weight: 300–350 g) were purchased from the Centre de Production Animale, Olivet, France. Animals were maintained on a standard diet in the animal facility of the UFR Biomédicale des Saints-Pères, Université Paris V, France. The animals were treated in accordance with the local Ethical Animal Care and Use Committee.

Antigen sources

All the NRL sources used in this study were the same as utilized previously.^{8,9}

The latex solution was prepared with a lyophilized non-ammoniated latex extract (C-serum) from a *Hevea brasiliensis* tree in Thailand. The lyophilized C-serum contained 57.2 µg latex antigenic proteins g⁻¹ of lyophilizate determined with the Competitive Immunoassay for Antigenic Latex Proteins (CIALP), an ELISA method for the assessment of antigenic NRL proteins described elsewhere.¹¹ For the preparation of NRL in saline, C-serum was reconstituted in saline solution and diluted to 0.5 µg NRL antigenic proteins ml⁻¹.

Latex-contaminated cornstarch (NRL-cornstarch) was collected in the slurry tank on the glove production line of a latex glove manufacturer in Thailand. Its antigenic NRL protein content was 0.85 µg g⁻¹ of dry powder (CIALP).

Virgin or non-contaminated cornstarch (Agenasorb 9020, Agrana Stärke GmbH, Wien, Austria) habitually used for powdering latex gloves that had never been in contact with NRL sources was utilized as a control for NRL-cornstarch.

Ovalbumin used for sensitization and challenge was a commercial reagent from Serlabo, France.

Sensitization protocol

The sensitization protocol was conducted exactly in the same manner as described previously.⁹ Guinea pigs, separated

in six groups ($n=8-10$ in each), were exposed to aerosolized NRL solubilized in saline or to NRL-cornstarch (10 µg of antigenic NRL proteins) once a day for 1 h for 2 weeks (2×5 days). The exposure was performed with or without a BB25 filter placed at the top side of the nebulizer. Control groups were exposed to saline alone or to non-contaminated cornstarch. All animals were challenged with NRL between days 31 and 33 of the sensitization protocol. Thirty minutes after bronchial challenge, the animals were bled and bronchoalveolar lavage (BAL) was performed.

Two additional groups were exposed to 10 µg ovalbumin every day as described above with (ovalbumin+filter group, $n=10$) or without a BB25 filter (ovalbumin group, $n=8$) inserted in the aerosol system. The ovalbumin bronchial challenge and BAL were performed according to the NRL sensitization schedule described above.

Specific bronchial provocation

The degree of bronchoconstriction after NRL or ovalbumin challenge was evaluated by the Konzett and Rössler method,¹² modified as described previously.⁸ Guinea pigs were anaesthetized with urethane intraperitoneally (1.5 g kg⁻¹), the right carotid artery was catheterized and connected to an arterial pressure transducer (DPT 700-500, NARCO Biosystems, Houston, TX, USA). The animals were tracheotomized and connected to an artificial respiration pump (Braun, Melsungen, Germany) and a differential pressure transducer (PM5, Statham, LA, CA, USA). The ventilation parameters (volume and frequency) for each animal were calculated according to the table of Kleinman and Radford.¹³ Anaesthetized animals were pre-treated with propranolol (2 mg kg⁻¹) (Sigma, St Louis, MO, USA) at least 10 min before challenge to prevent possible bronchodilatation induced by catecholamines.¹⁴ Challenge was then performed by instillation of 1 mg NRL or ovalbumin in 20 µl of saline solution directly into the lungs via the trachea. Pulmonary insufflation pressure variation and arterial pressure were recorded over 15 min with the differential pressure captor connected to a Physiograph E&M (NARCO Biosystems). The level of bronchoconstriction was determined after calculation of the area under the curve using the KaleidaGraph™ 3.09 software (Synergy Software, Reading, PA, USA).

Thromboxane B₂ determination in BAL fluid

Thirty minutes after NRL or ovalbumin challenge, BAL was performed as described below. Five millilitres of cold saline was instilled through the tracheal cannula then removed and re-injected five times with a 5 ml-syringe. The operation was repeated three times and the total volume of BAL fluid (BALF) was measured. BALF was centrifuged (15 min at 1000 g) and the supernatant stored at -20°C until assayed.

Thromboxane (Tx) B₂ was determined in BALF with a commercial kit supplied by Cayman Chemical Company (TxB₂ EIA kit, Ann Arbor, MI, USA). The assay was

performed according to the supplier's recommendations with a detection limit of 13 pg ml^{-1} .

Statistical analysis

Results were expressed as median (SEM) unless noticed otherwise. The Mann–Whitney *U*-test was used for comparisons between groups. A $P < 0.05$ was considered as statistically significant.

Results

Sensitization with NRL

Following bronchial challenge guinea pigs exposed to NRL without a filter (i.e. NRL alone and NRL-cornstarch) demonstrated higher level of bronchoconstriction compared with controls. Animals exposed in the presence of the BB25 filter exhibited an airway response similar to control (Fig. 1). Calculation of the area under the curve showed a level of bronchoconstriction significantly higher for animals exposed to NRL without a filter compared with control (respectively, $P < 0.02$ and $P < 0.001$ for NRL and NRL-cornstarch). Conversely, the level of bronchoconstriction in the groups exposed to NRL with the BB25 filter was not significantly different from control but significantly lower compared with those that have been treated without the filter ($P < 0.02$) (Table 1).

TxB₂ determination in BALF after bronchial challenge showed a significant increase of the production in the guinea pig lungs exposed to NRL ($P < 0.001$) compared with control.

When the animals were exposed in the presence of the filter we observed a decrease in TxB₂ production compared with those exposed to NRL without a filter ($P < 0.04$) (Table 2).

Sensitization with ovalbumin

Specific bronchial challenge performed after ovalbumin sensitization demonstrated an increase in bronchoconstriction compared with control group as shown by the area under the curve ($P < 0.001$). Exposure in the presence of a filter resulted in a significant decrease in the pulmonary response ($P < 0.001$ compared with ovalbumin), which was not different from control (Table 1 and Fig. 2).

TxB₂ production in lungs also significantly increased after exposure to ovalbumin ($P < 0.001$) compared with control. Exposure in the presence of the filter resulted in a significant decrease ($P < 0.04$) compared with the ovalbumin group (Table 2).

Discussion

In a recent study we developed an animal model of NRL sensitization in guinea pigs exclusively conducted via the respiratory route⁹. The animals were exposed to aerosolized NRL solubilized in saline or to NRL-contaminated cornstarch glove powder. These animals demonstrated respiratory sensitivity as shown by a high level of bronchoconstriction induced after NRL challenge and an increase of the TxB₂ production in the lungs. This model confirms that the respiratory tract is a predominant route of sensitization to NRL proteins. The allergens carried by

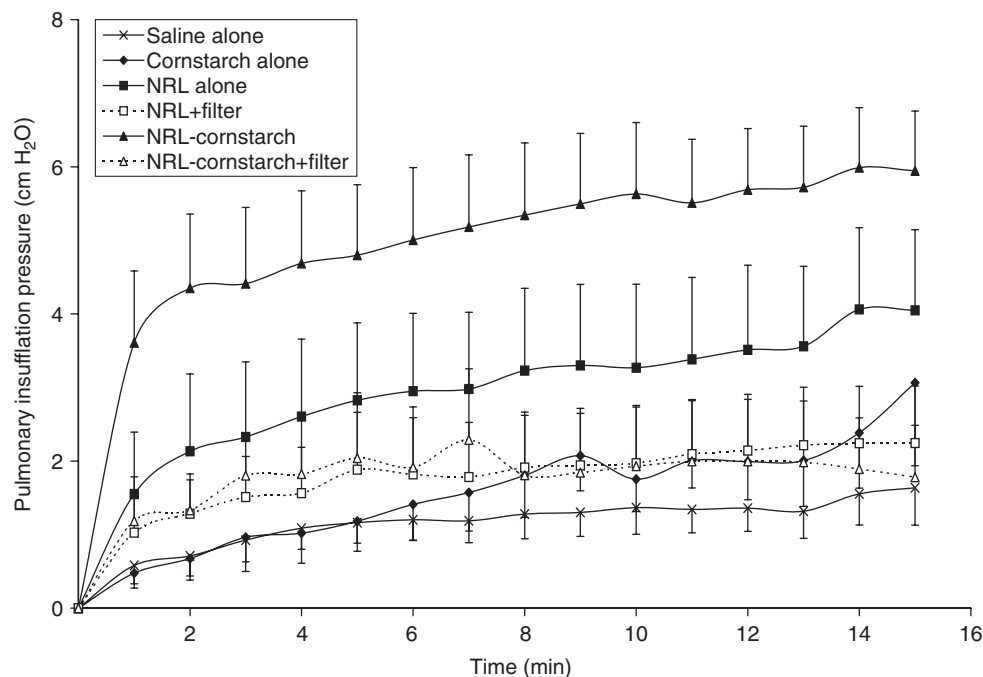


Fig 1 Pulmonary insufflation pressure variation in guinea pigs for 15 min following bronchial provocation with 1 mg NRL. Data are expressed as the mean of the group (SEM) ($n=8-10$ in each group).

Table 1 Area under the curve of the pulmonary insufflation pressure variations over 15 min for each guinea pig after NRL or ovalbumin bronchial challenge. Values are expressed in $\text{cmH}_2\text{O} \times \text{min}$. * $P < 0.02$ and ** $P < 0.001$ compared with controls. † $P < 0.02$ compared with NRL group. # $P < 0.02$ and ## $P < 0.005$ compared with the corresponding group treated without the BB25 filter

Guinea pigs	Exposed to							
	NRL alone	NRL+filter	Saline alone	NRL-cornstarch	NRL-cornstarch+filter	Cornstarch alone	Ovalbumin	Ovalbumin+filter
1	27.6	3.2	13.0	63.1	57.2	8.9	56.2	17.2
2	35.4	7.7	5.7	53.4	0.0	15.2	93.3	8.5
3	20.4	14.9	6.4	115.4	6.1	17.4	78.9	28.6
4	17.0	59.6	22.2	57.6	31.0	40.6	51.4	20.5
5	24.3	2.4	20.3	124.8	18.3	23.2	143.8	16.8
6	24.6	10.8	36.9	53.4	7.1	17.6	68.4	29.0
7	34.8	30.0	7.0	49.9	13.7	16.6	42.3	25.8
8	136.9	15.6	20.8	109.7	13.7	11.8	52.6	27.8
9	34.1		9.4	42.4	92.7	55.1		22.5
10	82.0		30.1			20.6		
Median (SEM)	30.9 (15.3)*	12.9 (7.7)#	16.7 (4.4)	57.6 (13.2)**†	13.7 (12.3)##	17.5 (5.8)	62.3 (12.5)**	22.5 (2.6)##

Table 2 Tx B₂ production in the lungs of guinea pigs exposed to NRL or ovalbumin, determined after NRL or ovalbumin challenge in BALF. Values are expressed in pg ml^{-1} . * $P < 0.001$ compared with the control groups. # $P < 0.04$ compared with the corresponding group treated without the BB25 filter

Guinea pigs	Exposed to							
	NRL alone	NRL+filter	Saline alone	NRL-cornstarch	NRL-cornstarch+filter	Cornstarch alone	Ovalbumin	Ovalbumin+filter
1	3144	1768	855	926	1163	87	2791	1909
2	3170	1637	2185	1450	1248	506	6290	1339
3	2785	1347	260	1964	567	295	1425	863
4	2399	625	890	731	738	298	1078	824
5	2120	449	117	1574	1376	892	3731	1842
6	1183	1142	339	1551	974	69	2309	1443
7	1874	4571	536	1208	1259	146	2287	636
8	3764	823	13	1924	1211	431	2309	1963
9	1541		804	1568	711	181		1086
10	1442		1317			362		
Median (SEM)	2260 (286)*	1245 (414)#	670 (217)	1551 (147)*	1163 (97)#	297 (82)	2309 (617)*	1339 (159)#

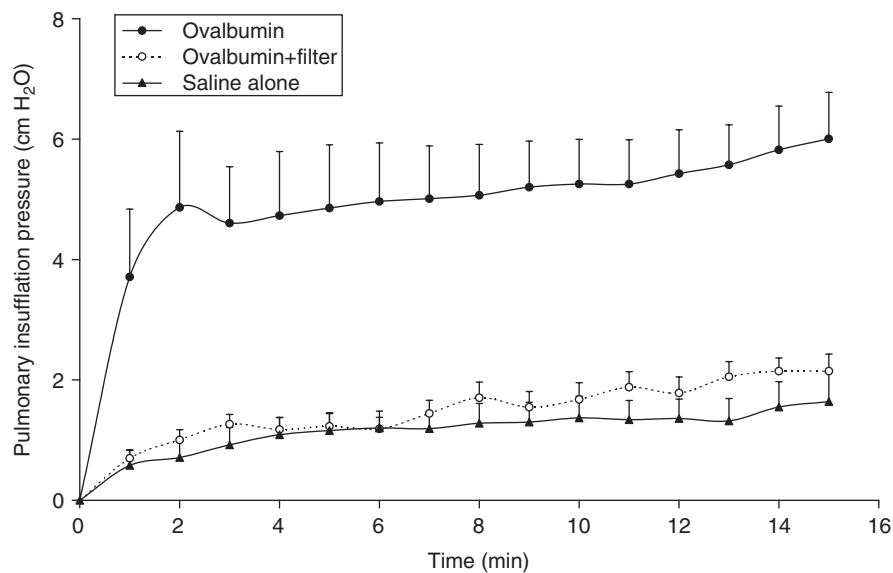


Fig 2 Pulmonary insufflation pressure variations in guinea pigs for 15 min following bronchial provocation with 1 mg ovalbumin. Data are expressed as the mean of the group (SEM) ($n=8-10$ in each group).

airborne glove cornstarch powder, contaminate the environment and can be inhaled. This explains why NRL-allergic health care workers (HCW) or patients can develop allergic reactions even though the use of gloves or other NRL-made devices is banned. We have also reported the role of cornstarch powder as an immunoadjuvant as airway responsiveness was increased in animals exposed to NRL-cornstarch compared with those treated with the same dose of NRL alone. The immunoadjuvant property of cornstarch in NRL-induced hypersensitivity was also demonstrated in a previous study in which guinea pigs were sensitized via the peritoneal route reproducing NRL sensitization of patients during surgical procedures.⁸

The present study corroborates these findings as exposure to NRL in the presence of the BB25 filter significantly decreased bronchoconstriction and TxB_2 production in lungs after bronchial challenge. The inflammatory mediator TxA_2 and its more stable metabolite TxB_2 are not allergen-specific but they are known to be the most potent bronchoconstrictors in guinea pig airways.¹⁵ Arakida and colleagues¹⁶ also reported that TxA_2 was the mediator predominantly found in the lungs of passively sensitized guinea pigs exhibiting asthmatic reactions.

The capacity for retention of cornstarch and NRL-cornstarch particles by the BB25 filter has already been tested.¹⁰ In the present experiments, we confirmed *in vivo* that the BB25 filter is able to protect guinea pigs from NRL sensitization during exposure to airborne NRL-cornstarch particles or NRL solubilized proteins. Animals exposed to NRL with the BB25 filter exhibited airway responses similar to those of the controls. Exposure to the well-known antigen ovalbumin supported these findings. The high variability in pulmonary insufflation pressure observed may be the result of a variation of the dose of allergen effectively received. Guinea pigs were allowed to breathe spontaneously during the sensitization protocol, thus the dose of the NRL inhaled was not controlled. This variability was also observed in animals sensitized to ovalbumin.

NRL is known to be responsible for anaphylactic reactions in patients during surgical procedures in 16.6% of the cases. The severity of these reactions underlines that pre-anaesthetic detection of the triggering parameters is very important and the implementation of preventative measures is essential. Recent reports re-inforced the need to take measures to efficiently protect patients and/or HCW against NRL exposure.^{17,18} The key elements are the establishment of an as close as possible latex-free environment and minimizing the exposure to latex either in primary or secondary prophylaxis.^{19,20} For example, in a 5-yr prospective study on 100 children mostly with spina bifida, the anti-latex IgE was reduced in 64% of the patients by applying these procedures.²¹ A notable efficiency in the HCW population was also observed.^{22,23}

As a result of the latex risk, NRL-made devices have been discarded from the operating theatre. However, in many hospitals, mechanical ventilation is still provided by

older equipment with components containing NRL. The wear of such apparatus can lead to latex scatter within the piping, which can be inhaled by the patient. Nevertheless, the recommendations mentioned above are strengthened when a patient at risk is anaesthetized even when using a newer breathing system. Hence, some anaesthetists require that the viral and bacterial filter be inserted between the Y-piece and the patient to efficiently retain latex particles.

The BB25 filter has been developed for anaesthesia, it retains small airborne bacterial and viral contaminants with a retention efficiency of more than 99.999% (*Brevundimonas diminuta*, Bacteriophage MS2) and larger particles (*Bacillus subtilis*, *Serratia marcescens*) with a retention efficiency of more than 99.9999%. Its hydrophobic membrane prevents the passage of aqueous suspension of *Mycobacterium bovis*, *Mycobacterium tuberculosis*, HCV and HIV. The present study demonstrated that the use of this filter should contribute efficiently to the patient protection during surgery or any procedure necessitating mechanical ventilation. However, all other measures intended to avoid contact between the patient and NRL devices must be taken. Nakamura and colleagues²⁴ reported a high incidence of latex allergy in children requiring home mechanical ventilation.

Mitakakis and colleagues²⁵ have tested the efficiency of a particulate mask to protect HCW from airborne latex allergens. But to our knowledge, this is the first study of a device used to prevent latex-allergy reactions in patients.

Acknowledgement

Financial support from Pall France, Saint-Germain-en-Laye, France, who manufactures the BB25 filter is gratefully acknowledged.

References

- 1 Lundberg M, Wrangsjö K, Johansson SGO. Latex allergy from glove powder—an unintended risk with the switch from talc to cornstarch? *Allergy* 1997; **52**: 1222–8
- 2 Levy DA, Allouache S, Chabane MH, Leynadier F, Burney P. Powder-free protein-poor natural rubber latex gloves and latex sensitization. *J Am Med Assoc* 1999; **281**: 988
- 3 Sussman GL, Liss GM, Deal K, et al. Incidence of latex sensitization among latex glove users. *J Allergy Clin Immunol* 1998; **101**: 171–8
- 4 Newsom SWB, Shaw M. A survey of starch particles in the hospital environment in relation to the use of powdered latex gloves. *Occup Med* 1997; **47**: 155–8
- 5 Kujala V, Alenius H, Palosuo T, Karvonen J, Pääffli P, Reijula K. Extractable latex allergens in airborne glove powder and in cut glove pieces. *Clin Exp Allergy* 2002; **32**: 1077–81
- 6 Laxenaire MC, Mertes PM, Groupe d'Etudes des Réactions Anaphylactoides Peranesthésiques. Anaphylaxis during anaesthesia. Results of a two-year survey in France. *Br J Anaesth* 2001; **87**: 549–58
- 7 Rüeff F, Kienitz A, Schöpf P, et al. Frequency of natural rubber latex allergy in adults is increased after multiple operative procedures. *Allergy* 2001; **56**: 889–94
- 8 Barbara J, Santais M-C, Levy DA, Ruff F, Leynadier F. Immunoadjuvant properties of glove cornstarch powder in latex-induced hypersensitivity. *Clin Exp Allergy* 2003; **33**: 106–12

- 9 Barbara J, Santais M-C, Levy DA, Ruff F, Leynadier F. Inhaled cornstarch glove powder increases latex-induced airway hypersensitivity in guinea pigs. *Clin Exp Allergy* 2004; **34**: 978–83
- 10 Barbara J, Chabane MH, Leynadier F, Girard F. Retention of airborne latex particles by a bacterial and viral filter used in anaesthesia. *Anaesthesia* 2001; **56**: 231–4
- 11 Chabane H, Barbara J, Hrabina M, Leynadier F. Competitive immunoassay for antigenic latex protein measurement: Rabbit antiserum-based assay compared to modified Lowry and human IgE-inhibition methods. *J Invest Allergol Clin Immunol* 1999; **9**: 372–9
- 12 Konzett H, Rössler R. Versuchsanordnung zu untersuchungen an der bronchialmuskulatur. *Arch Exp Path Pharmacol* 1940; **195**: 71–4
- 13 Kleinman LI, Radford EP. Ventilation standards of small mammals. *J Appl Physiol* 1964; **19**: 360–2
- 14 Lau WAK, Rechtman MP, Boura ALA, King RG. Synergistic potentiation by captopril and propranolol of bradykinin-induced bronchoconstriction in the guinea pig. *Clin Exp Pharmacol Physiol* 1989; **16**: 599–602
- 15 Kleeberger SR, Freed AN. 65. Prostanoids. In Busse WW, Holgate ST, eds. *Asthma and Rhinitis*, 2nd edn. New York: Blackwell Science Ltd, 1999; 1: 999–1013
- 16 Arakida Y, Ohga K, Suwa K, *et al.* Effects of lipid mediator antagonists on predominant mediator-controlled asthmatic reactions in passively sensitized guinea pigs. *J Pharmacol Exp Ther* 1999; **290**: 1285–91
- 17 Elliott BA. Latex allergy: the perspective from the surgical suite. *J Allergy Clin Immunol* 2002; **110**: S117–20
- 18 Lieberman P. Anaphylactic reactions during surgical and medical procedures. *J Allergy Clin Immunol* 2002; **110**: S64–9
- 19 Cremer R, Kleine-Diepenbruck U, Hoppe A, Bläker F. Latex allergy in spina bifida patients—prevention by primary prophylaxis. *Allergy* 1998; **53**: 709–11
- 20 Liss GM, Tarlo SM. Natural rubber latex-related occupational asthma: association with interventions and glove changes over time. *Am J Industrial Med* 2001; **40**: 347–53
- 21 Reider N, Kretz B, Menardi G, Ulmer H, Fritsch P. Outcome of a latex avoidance program in a high-risk population for latex allergy—a five-year follow-up study. *Clin Exp Allergy* 2002; **32**: 708–13
- 22 Hamilton RG, Brown RH. Impact of personal avoidance practices on health care workers sensitized to natural rubber latex. *J Allergy Clin Immunol* 2000; **105**: 839–41
- 23 Allmers H, Schmengler J, Skudlik C. Primary prevention of natural rubber latex allergy in the German health care system through education and intervention. *J Allergy Clin Immunol* 2002; **110**: 318–23
- 24 Nakamura CT, Ferdman RM, Keens TG, Davidson Ward SL. Latex allergy in children on home mechanical ventilation. *Chest* 2000; **118**: 1000–3
- 25 Mitakakis TZ, Tovey ER, Yates DH, *et al.* Particulate masks and non-powdered gloves reduce latex allergen inhaled by healthcare workers. *Clin Exp Allergy* 2002; **32**: 1166–9