

Inhaled cornstarch glove powder increases latex-induced airway hyper-sensitivity in guinea-pigs

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Summary

Background Breathing is one of the most important modes of sensitization to natural rubber latex (NRL) for health-care workers, a group most at risk. Cornstarch powder (CSp) from medical powdered NRL gloves is known to be an allergen carrier, and sensitization to NRL can occur by inhaling airborne particles from such gloves.

Objective The aim of this study was to demonstrate, using an experimental model, which CSp may act as an adjuvant in NRL-induced airway hyper-responsiveness.

Methods Guinea-pigs were exposed to aerosolized NRL-contaminated CSp or to NRL in saline solution for 1 h every day for 2 weeks. The control groups were exposed either to CSp or to saline alone. An additional group of guinea-pigs was exposed to aerosolized ovalbumin (OVA) in saline. Three weeks after the last exposure, specific bronchial challenges were performed. In addition, Specific IgG and IgG1 in sera and thromboxane (Tx) B₂ levels in bronchoalveolar lavage fluid (BALF) were measured.

Results The NRL challenge caused significant bronchospasm in the animals that had been exposed to NRL compared with those in the control groups ($P < 0.02$). Guinea-pigs exposed to OVA also demonstrated a significant bronchospasm after OVA challenge ($P < 0.001$). The guinea-pigs that had inhaled NRL-contaminated CSp had a significantly higher bronchoconstriction level than those that had inhaled NRL alone ($P < 0.02$). Specific IgG and IgG1 were undetectable in sera from all groups, whereas significant amounts of Tx B₂ ($P < 0.001$) were found in the lungs of the guinea-pigs exposed to NRL or OVA.

Conclusion Inhaling CSp increases the airway response to NRL. The fact that specific IgG and IgG1 were not detected might be the result of an immune response limited to the airways. This finding is supported by a significant increase of Tx B₂ level in the BALF of sensitized guinea-pigs.

Keywords asthma, bronchoalveolar lavage, bronchoconstriction, guinea-pig, latex, thromboxane

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Introduction

Natural rubber latex (NRL) allergy has become an important occupational health hazard during the last two decades. Health-care workers (HCW) are one of the groups most at risk because of the extensive use of NRL devices, such as powdered NRL medical gloves, to protect both patients and HCW. Direct skin contact may be the predominant route of NRL sensitization for HCW but inhalation of airborne glove powder bearing NRL proteins has also been shown to lead to sensitization [1–4]. Glove cornstarch powder (CSp) bearing latex allergens contaminates the workplace environment and can induce allergic symptoms in latex-allergic individuals. A correlation between the use of powdered NRL gloves and latex sensitization has been reported [5, 6]. The switch from

talc to CSp glove donning lubricant may be one of the causes of the increase in the prevalence of NRL allergy: CSp particles are more volatile than talc and adsorb greater amounts of NRL protein [7]. Furthermore, a significant correlation between NRL aeroallergens and airborne CSp dust has been reported recently [8]. Thus, we have to consider NRL-contaminated CSp as a potent aeroallergen source [9].

NRL has been shown to be able to induce pulmonary reactions in guinea-pigs as well as in other experimental models [10–13]. It has also been shown that the route of sensitization can lead to different reaction patterns [14].

In a previous report, we had demonstrated that CSp acts as an immunoadjuvant in guinea-pigs sensitized to NRL by the peritoneal route [15]. In the present study, our aim was to test the hypothesis that CSp could potentiate the immunogenicity of NRL through breathing latex-bearing CSp. We sensitized guinea-pigs to NRL by having them breathe CSp bearing NRL protein. Guinea-pigs were used because they are a very good model for evaluating pulmonary responses to inhaled allergens. Furthermore, the morphology and reactions of

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their respiratory tract are very similar to those of human beings [16–18]. Our results support the hypothesis that the use of powdered latex gloves can induce NRL hyper-sensitivity *via* airway sensitization, as previously demonstrated in epidemiological studies [5, 6].

Materials and methods

Animals

Male albino Dunkin–Hartley guinea-pigs (weight: 300–350 g) were purchased from the Centre de Production Animale, Olivet, France. They were fed 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

The NRL source was a lyophilized C-serum from non-ammoniated latex, obtained directly from a *Hevea brasiliensis* tree in Thailand and prepared as described previously [19]. The C-serum contained 240 mg protein/g of lyophilizate, determined by the modified Lowry method according to the EN 455-3 European norm [20].

Two CSp sources were used: (1) virgin CSp (VCSp), generally used as glove lubricant (Agenasorb 9020, Agrana Stärke GmbH, Wien, Austria), never exposed to any NRL source and (2) latex-contaminated CSp (NRL-CSp), containing 1324 µg of protein/g of dry powder (EN 455-3), collected from the slurry tank in a glove production line in Thailand. We used NRL-CSp to sensitize the guinea-pigs in order to make our model resemble reality as much as possible and to reproduce the conditions of exposure to NRL in workplaces such as health-care centres where powdered latex gloves are used.

C-serum, VCSp and NRL-CSp samples were previously assayed by the CIALP method (competitive immunoassay for antigenic latex proteins) for the determination of antigenic NRL protein content [21]. In order to expose all the experimental groups to the same dose of NRL, the C-serum was diluted to 0.5 µg of antigenic NRL protein/mL in saline and used as the latex aerosol solution. Endotoxin activity was evaluated in VCSp and NRL-CSp using the kinetic chromogenic quantitative *Limulus amoebocyte* lysate assay (Bio Whittaker Europe SPRL, Verviers, Belgium) according to the European Pharmacopoeia. Endotoxin content was 1.3 ng/g of cornstarch for VCSp and 324 ng/g for NRL-CSp [15].

Sensitization protocol

Animals, separated in four groups ($n = 10$ each), were sensitized by repeated exposure to aerosolized antigen for 2 weeks (2×5 days) (Fig. 1). The NRL alone group and the NRL-CSp group were exposed daily to 10 µg of NRL antigenic proteins in 20 mL saline or 12 g of CSp, respectively. The control groups, that is, VCSp and saline alone, were exposed to 12 g of VCSp or 20 mL saline, respectively. The animals were wrapped in cotton sleeves to avoid any skin contact with aerosolized NRL, then placed in a Plexiglas chamber (volume: 8.8 L). NRL antigens in solution or in NRL-CSp were placed in a nebulizer (Misty Neb Air Life,

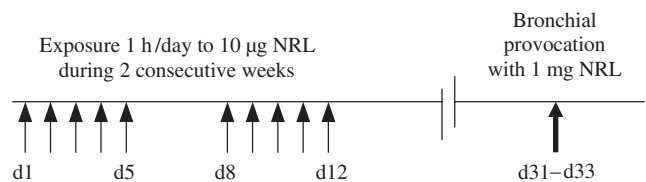


Fig. 1. Sensitization procedure. Guinea-pigs divided in four groups ($n = 10$ in each) were exposed 1 h/day during 2 consecutive weeks (d1 to d5 and d8 to d12) to aerosolized NRL-contaminated cornstarch powder (NRL-CSp), virgin (non-contaminated) cornstarch powder (VCSp), NRL in saline solution or saline alone. The animals exposed to NRL-CSp or NRL alone were given the same dose of NRL proteins (10 µg/day). NRL-CSp and VCSp groups were exposed to the same cornstarch powder amount (12 g/day). Between d30 and d33 of the sensitization protocol, all the animals were challenged with 1 mg NRL. Blood and BALF were collected 30 min after challenge.

Allegiance, McGaw Park, IL, USA) and dispersed into the chamber by means of compressed air (8 L/min, ECONONEb, Medix, Mediflux, Bry-sur-Marne, France). In a previous study in which we exposed guinea-pigs to 1 g NRL-CSp for only 10 min/day, no significant airway responses were observed compared with control animals (data not shown). For this reason, we increased the dose and time of exposure to 12 g NRL-CSp for 1 h/day.

All animals were challenged with NRL in the third week after sensitization, between days 31 and 33. Thirty minutes after bronchial challenge, the animals were bled and bronchoalveolar lavage fluid (BALF) was obtained.

One additional group ($n = 8$) was sensitized to aerosolized ovalbumin in saline (OVA, Serlabo, France). Exposure and challenge with OVA were performed in the manner described above.

Specific bronchial provocation

The degree of bronchoconstriction (BC) after NRL or OVA challenge was evaluated by the method of Konzett and Rössler [22] and modified as previously described [15]. In brief, guinea-pigs were anaesthetized with urethane (1.5 g/kg) intraperitoneally, tracheotomized and pre-treated with propranolol (2 mg/kg, i.p.) (Sigma, Saint-Louis, MO, USA) at least 10 min before the challenge to prevent possible bronchodilatation induced by catecholamines [23]. Challenges were performed by instilling 1 mg of antigenic NRL or 1 mg OVA in 20 µL of saline solution directly into the guinea-pig lungs *via* the trachea. Variations in pulmonary insufflation pressure were then recorded for 15 min. BC levels were estimated after calculation of the area under the curve.

Determination of specific IgG and IgG1

Anti-NRL and anti-OVA IgG and IgG1 in serum and in BALF collected from each guinea-pig 30 min after bronchial challenge were determined by ELISA, as previously described [15]. A peroxidase-labelled rabbit anti-guinea-pig IgG (Sigma) was used for the IgG determination assay, and IgG1 was determined using a rabbit anti-guinea-pig IgG1 (heavy chain-specific, Bethyl Laboratories, Montgomery, TX, USA) and a peroxidase-labelled mouse monoclonal anti-rabbit (Sigma).

Prostaglandin $F_{2\alpha}$ and thromboxane B_2 determination in BALF

BAL was performed thirty min after NRL or OVA challenge. Using a 5 mL-syringe, 5 mL of cold saline solution were instilled into the lungs through the tracheal cannula then retrieved by aspiration and re-injected five times. The operation was repeated three times with, every time, 5 mL of new saline solution. The total volume of BALF was measured, centrifuged (15 min at 1000 g) and the supernatant was stored at -20°C until assayed.

Prostaglandin (Pg) $F_{2\alpha}$ and thromboxane (Tx) B_2 were determined in BALF with commercial kits supplied by the Cayman Chemical Company, Ann Arbor, MI, USA ($\text{PgF}_{2\alpha}$ and TxB_2 EIA kits). The assays were performed according to the supplier's recommendations.

Statistical analysis

All results were expressed as mean $\pm$ SEM. Area under the curve was calculated with KaleidaGraphTM 3.09 software (Synergy Software, Reading, PA, USA). The Mann-Whitney U -test was used for comparisons between groups. Correlations were calculated using the non-parametric Spearman correlation test. $P < 0.05$ was considered as statistically significant.

Results

Specific bronchial challenge

Both groups of guinea-pigs exposed to NRL, either in saline or adsorbed on CSp, showed larger variations in pulmonary insufflation pressure after latex challenge than the control groups (Fig. 2). Thus, a significant BC was induced in the NRL-CSp and NRL alone groups after latex challenge. The area under the curve for each animal is shown in Fig. 3.

Significant airway hyper-responsiveness was observed in guinea-pigs previously exposed to NRL-CSp ($74.40 \pm 10.79 \text{ cm cmH}_2\text{O} \times \text{min}$, $P < 0.001$) or NRL alone ($43.70 \pm 11.86 \text{ cm cmH}_2\text{O} \times \text{min}$, $P < 0.02$) compared with the controls (saline alone: $17.19 \pm 3.39 \text{ cm cmH}_2\text{O} \times \text{min}$ and VCSp: $22.70 \pm 4.51 \text{ cm cmH}_2\text{O} \times \text{min}$). Moreover, animals exposed to CSp bearing NRL proteins had significantly greater BC response compared with those exposed to NRL in solution ($P < 0.02$).

In addition, the OVA group also showed a significant increase in BC response after OVA challenge compared with the control group ($73.35 \pm 12.45 \text{ cm cmH}_2\text{O} \times \text{min}$, $P < 0.001$) (Figs 2 and 3).

Specific IgG and IgG1 determination

Neither specific IgG nor specific IgG1 were detected in the sera and BALF obtained from the guinea-pigs.

TxB_2 and $\text{PgF}_{2\alpha}$ determination in BALF

Significant amounts of TxB_2 were found in BALF of guinea-pigs exposed to NRL antigens (NRL alone: $2342 \pm 286 \text{ pg/mL}$ and NRL-CSp: $1432 \pm 147 \text{ pg/mL}$, $P < 0.001$), compared with the controls (saline alone: $732 \pm 217 \text{ pg/mL}$ and VCSp: $326 \pm 82 \text{ pg/mL}$). The TxB_2 level was greater for the NRL alone group than for the NRL-CSp group ($P < 0.05$) (Fig. 4). Significant $\text{PgF}_{2\alpha}$ levels were observed in the NRL alone group ($1098 \pm 164 \text{ pg/mL}$, $P < 0.05$) compared with the controls (saline alone: $583 \pm 168 \text{ pg/mL}$ and VCSp: $443 \pm 116 \text{ pg/mL}$) but not in the NRL-CSp group ($381 \pm 114 \text{ pg/mL}$) (Fig. 4).

The OVA group exhibited a significant increase of TxB_2 level in BALF compared with the controls ($2778 \pm 617 \text{ pg/mL}$, $P < 0.001$) but not in $\text{PgF}_{2\alpha}$ ($203 \pm 60 \text{ pg/mL}$).

A significant correlation was found between the BC level and TxB_2 concentration ($r = 0.41$; $P = 0.012$) but not with $\text{PgF}_{2\alpha}$ ($r = 0.15$; $P = 0.374$).

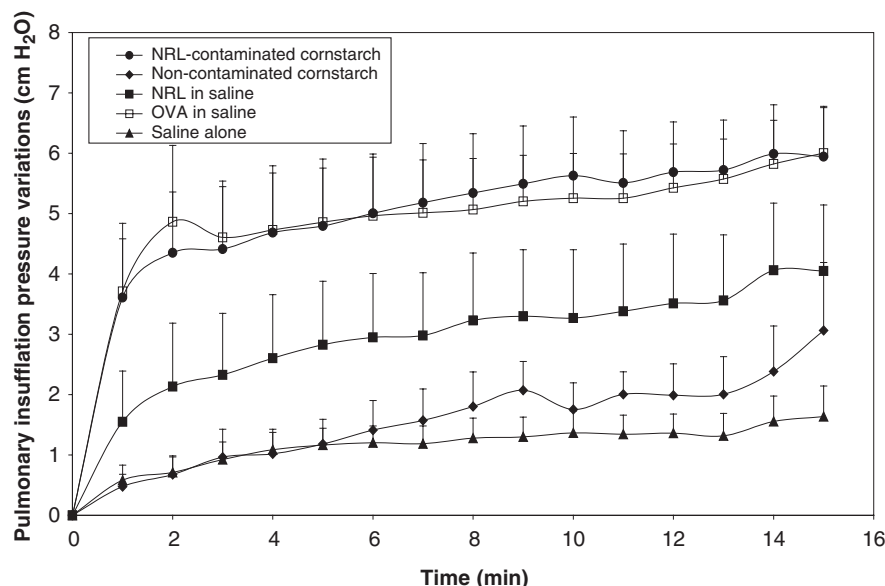


Fig. 2. Pulmonary insufflation pressure variations measured in guinea-pigs for 15 min after bronchial provocation. Data are expressed as the mean of the group $\pm$ SEM. Guinea-pigs ($n = 10$ in each group) were previously exposed to NRL-contaminated cornstarch powder (NRL-CSp), virgin (non-contaminated) cornstarch powder (VCSp), NRL in saline or saline alone. Challenge was performed with 1 mg of NRL. Ovalbumin (OVA) group ($n = 8$) was exposed to OVA in saline and challenged with 1 mg OVA.

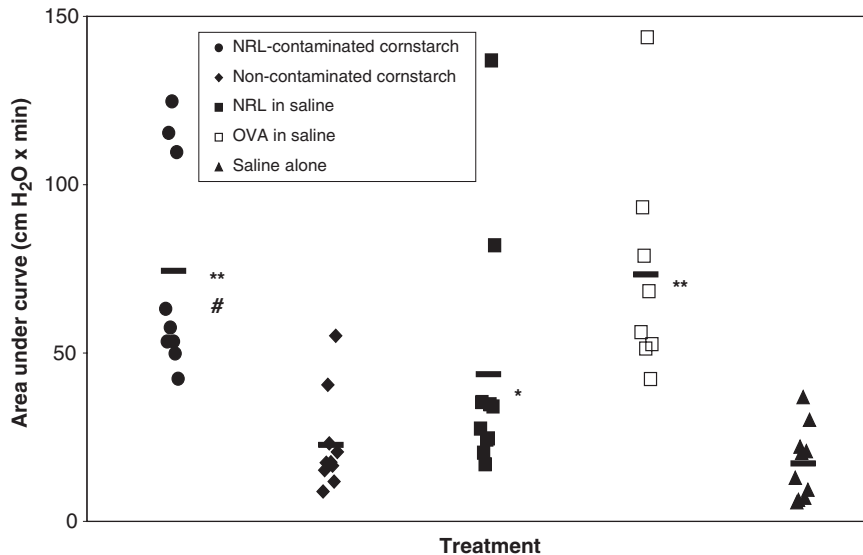


Fig. 3. Bronchoconstriction level expressed as the area under the curve calculated for each guinea-pig after bronchial provocation. The mean of each group is represented as the '—' symbol. Guinea-pigs ($n = 10$ in each group) were previously exposed to NRL-contaminated cornstarch powder (NRL-CSp), virgin (non-contaminated) cornstarch powder (VCSp), NRL in saline solution or saline alone. Challenge was performed with 1 mg of NRL. Ovalbumin (OVA) group ($n = 8$) was exposed to OVA in saline and challenged with 1 mg OVA. * $P < 0.02$ and ** $P < 0.001$ compared with control groups, # $P < 0.02$ compared with NRL alone group.

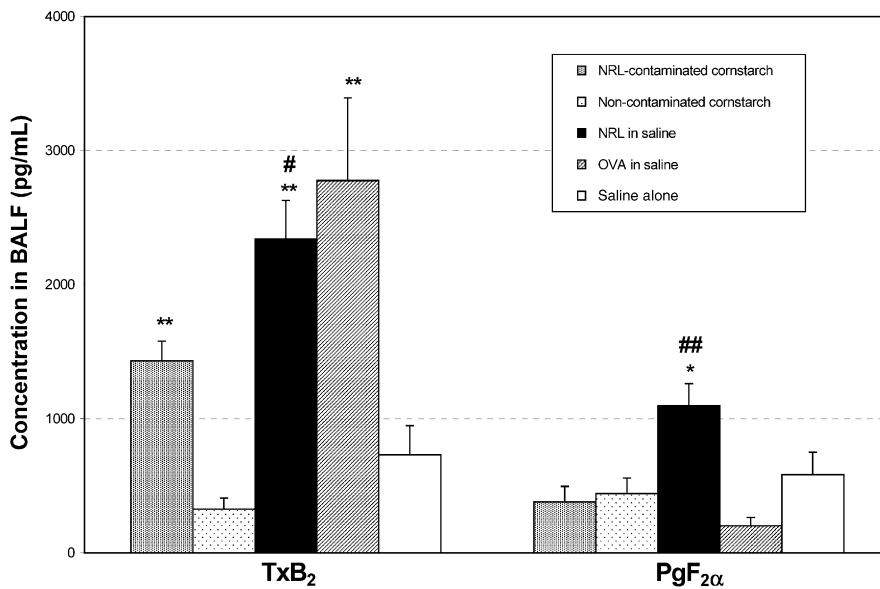


Fig. 4. Thromboxane B₂ (TxB₂) and prostaglandin F_{2α} (PgF_{2α}) amounts in bronchoalveolar lavage fluid (BALF) of guinea-pigs after bronchial challenge. Data are expressed as the mean of the group $\pm$ SEM. Guinea-pigs ($n = 10$ in each group) were previously exposed to NRL-contaminated cornstarch powder (NRL-CSp), virgin (non-contaminated) cornstarch powder (VCSp), NRL in saline solution or saline alone. Ovalbumin (OVA) group ($n = 8$) was exposed to OVA in saline. * $P < 0.05$ and ** $P < 0.001$ compared with the control groups, # $P < 0.05$ and ## $P < 0.01$ compared with NRL-CSp group.

Discussion

An experimental model of NRL-induced hyper-sensitivity was developed for this study. Sensitization was achieved exclusively *via* the respiratory route using NRL in solution or NRL-contaminated CSp. We demonstrated clearly that inhaling NRL repeatedly can lead to respiratory sensitivity as shown by the BC induced by specific bronchial provocation and increased recovery of the inflammatory mediator TxB₂. Furthermore, inhalation of NRL-contaminated CSp particles induced a greater airway responsiveness than did sensitization with NRL protein alone. These findings confirm those observed in a previous study in which we sensitized guinea-pigs to NRL-contaminated CSp *via* the peritoneal route, which reproduced the conditions of exposure to NRL of patients during surgical procedures. Guinea-pigs sensitized

with NRL-contaminated CSp intraperitoneally had demonstrated more BC and latex-specific antibody than those treated with NRL alone [15]. In addition, guinea-pigs exposed to OVA also exhibited significant BC after OVA challenge. Exposition to OVA in the presence of CSp was not explored, but in the study cited above, we had already demonstrated that sensitization with OVA+CSp by the peritoneal route resulted in similar respiratory and humoral responses after sensitization with NRL+CSp.

In our previous study, we found that there was 1.3 ng/g of cornstarch of endotoxin in the VCSp and 324 ng/g of cornstarch in the NRL-CSp we had used [15]. The same lots of CSp and NRL were used in the present study and in those cited above, but in the previous study the animals had been sensitized with 1 g of CSp, whereas in the present experiment they were exposed to a total of 120 g CSp. Sensitization with

different doses of CS_p and thus different doses of endotoxin resulted in similar respiratory responses, that is, increased airway responsiveness after challenge. Recent reports mention the discrepancies observed in animal studies concerning the role of lipopolysaccharides in allergic responses [24, 25]. Large doses of lipopolysaccharides can lead to the development of a Th1 response whereas low doses tend to result in a Th2 response. With regards to the results obtained after sensitization either by the peritoneal or the respiratory route, the role of endotoxin did not seem to be relevant in the present experiments. We believe that it was the CS_p acting as an immunoadjuvant in NRL sensitization.

Latex-specific antibodies were not found either in the sera or the BALF of the guinea-pigs. In previous studies, low to undetectable specific antibody production was found after airway sensitization to mites or moulds [26, 27]. Nabe et al. observed a significant increase in airway resistance 3 weeks after guinea-pigs were sensitized to cedar pollen through the respiratory route. However, specific antibodies were not observed until 8 weeks after sensitization [28]. According to these findings, specific antibodies must have appeared later than the respiratory response. Because we stopped the present study 3 weeks after sensitization, this might explain the fact that we detected no circulating antibodies.

Chvatchko et al. reported local production of specific IgE in the lungs of mice sensitized to OVA by multiple intratracheal instillations [29]. Local expression of mRNA of the IgE ϵ heavy chain has also been found in the respiratory tract of atopic and non-atopic asthma patients [30]. The fact that antibodies were not detected in the BALF may be due to the relative rapidity with which it was collected, that is, 30 min after challenge. Thus, the antibodies might have been complexed with the antigen. Further studies, such as determination of histological features of the lungs, are needed.

Nevertheless, the respiratory response we observed was specific for NRL proteins, since guinea-pigs exposed to non-contaminated CS_p alone did not react after NRL challenge, whereas those sensitized with NRL-contaminated CS_p responded with most BC. Thus, the guinea-pigs developed an asthmatic response, limited to the airways, after NRL challenge.

The detection of inflammatory mediators in BALF confirms the notion that exposure to NRL can lead to airway obstruction. Tx_{A2} and its more stable metabolite Tx_{B2} are known to be the most potent bronchoconstrictors in guinea-pigs airways [31]. It has also been reported that Tx_{A2} is the predominant mediator found in the lungs of passively sensitized guinea-pigs exhibiting asthmatic reactions [32]. In the present study, production of Tx_{B2} rather than PgF_{2 α} was observed. NRL-contaminated CS_p only induced Tx_{B2} production, whereas exposure to NRL in saline induced a significant increase of both PgF_{2 α} and Tx_{B2}. We do not have any satisfactory explanation for these results but the nature of the inhaled antigen (i.e. solid particles or soluble aerosols) does not seem to be relevant since inhaling OVA in saline solution only induced Tx_{B2} production.

In conclusion, our results suggest that CS_p plays a dual role in NRL-induced hyper-sensitivity. Its role as an allergen carrier has already been established. Here, we demonstrate that CS_p also acts as an adjuvant since it increases the airway

responsiveness of NRL-sensitized guinea-pigs. Because sensitization to NRL proteins is still an important occupational hazard, we recommend that the use of powdered gloves be restricted and that, in addition, respiratory exposure to airborne NRL be reduced.

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