Cutaneous, But Not Airway, Latex Exposure Induces Allergic Lung Inflammation and Airway Hyperreactivity in Mice

Maili Lehto,*[1] Rita Haapakoski,*[1] Henrik Wolff,*†‡ Marja-Leena Majuri,* Mika J. Mäkelä,§ Marina Leino,* Timo Reunala,¶ Kristiina Turjanmaa,¶ Timo Palosuo,# and Harri Alenius,*

*Departments of Industrial Hygiene and Toxicology and Occupational Medicine, Finnish Institute of Occupational Health, Helsinki, Finland; †Department of Pathology, Kymenlaakso Central Hospital, Kotka, Finland; ‡Department of Pathology, Helsinki University Central Hospital, Helsinki, Finland; §Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland; ¶Department of Dermatology, Tampere University and University Hospital, Tampere, Finland; #Laboratory of Immunobiology, National Public Health Institute, Helsinki, Finland

As respiratory symptoms are common in addition to skin reactions in natural rubber latex allergy, we investigated the significance of different allergen exposure routes in the development of lung inflammation and airway hyperreactivity (AHR). Both intracutaneous (IC) and intraperitoneal (IP) exposure followed by airway challenge with latex proteins induced an influx of mononuclear cells and eosinophils to the lungs. AHR and lung mucus production increased significantly after IC and IP but not after intranasal (IN) exposure. Infiltration of inflammatory cells was associated with the induction of T-helper type 2 (Th2) cytokines and several CC chemokines. Only a marginal induction of these mediators was found after IN exposure. On the contrary, increased levels of transforming growth factor-β1 and forkhead box 3 mRNA, markers of regulatory activities, were found in the lungs after IN but not after IC exposure. Finally, IC and IP, but not IN, latex exposure induced a striking increase in specific immunoglobulin E (IgE) levels. Cutaneous latex exposure in the absence of adjuvant followed by airway challenge induces a local Th2-dominated lung inflammation and a systemic IgE response. Cutaneous exposure to proteins eluting from latex products may therefore profoundly contribute to the development of asthma in latex allergy.

Key words: asthma/chemokines/cytokines/latex hypersensitivity
J Invest Dermatol 125:962–968, 2005

Natural rubber latex (NRL) allergy has been recognized as a major cause of occupational contact urticaria, rhinitis, and asthma in health care workers (Ahmed *et al*, 2003; Reunala *et al*, 2004). People in other occupations requiring the use of latex gloves or who are frequently in contact with other NRL products are also at risk of becoming sensitized to NRL (Turjanmaa and Makinen-Kiljunen, 2002; Ahmed *et al*, 2003; Sparta *et al*, 2004). Although skin is the most frequently reported site of allergic reactions from latex gloves, airway symptoms are also common (Fish, 2002; Nolte *et al*, 2002). In a recent study, one-fourth of the latex-allergic health care workers were reported to suffer from rhinitis and asthma and several of these asthmatic individuals were forced to change their jobs (Bernstein *et al*, 2003). Sensitization routes and pathomechanisms of NRL-induced rhinitis and asthma, however, are still poorly understood.

Sensitization to NRL proteins may occur through various exposure routes (Weissman and Lewis, 2002). Skin is an obvious target for exposure to latex proteins in people using latex gloves (Bernstein *et al*, 2003) but NRL allergens also become easily airborne in association with glove powder, thereby permitting exposure and sensitization via the airways (Charous *et al*, 2002). On the other hand, children with spina bifida are thought to become sensitized through mucous membranes to NRL proteins eluting from surgeon's gloves during operations (Weissman and Lewis, 2002). Today, little is known on how these different exposure routes interact during the development of NRL allergy or what is their importance in the elicitation of clinical symptoms.

In this study, we used mouse models of latex allergy to investigate the significance of different exposure routes, i.e. intracutaneous (IC), intraperitoneal (IP), and intranasal (IN) routes, to the development of lung inflammation, airway hyperreactivity (AHR), and antibody production to NRL allergens.

Results

IC exposure and challenge with NRL induces a vigorous influx of eosinophils into the lungs H&E staining of lung tissues revealed enhanced peribronchial and perivascular cell infiltrates consisting primarily of lymphocytes and eosinophils after IC and IP exposure (Fig 1*A* and S1). Only a slight, mostly perivascular, inflammation was observed after IN exposure. The number of infiltrating eosinophils both in perivascular and in peribronchial areas of lung tissues showed a dramatic increase in intracutaneously

Abbreviations: AHR, airway hyperreactivity; BAL, bronchoalveolar lavage; Foxp3, forkhead box 3; Hev b6.01, prohevein; IC, intracutaneous; IgE, immunoglobulin E; IL, interleukin; IN, intranasal; IP, intraperitoneal; MCh, methacholine; NRL, natural rubber latex; PAS, periodic acid-Schiff; PBS, phosphate-buffered saline; Penh, enhanced pause; RU, relative units; TGF-β1, transforming growth factor-β1; Th2, T-helper type 2

[1]These authors contributed equally to this work.

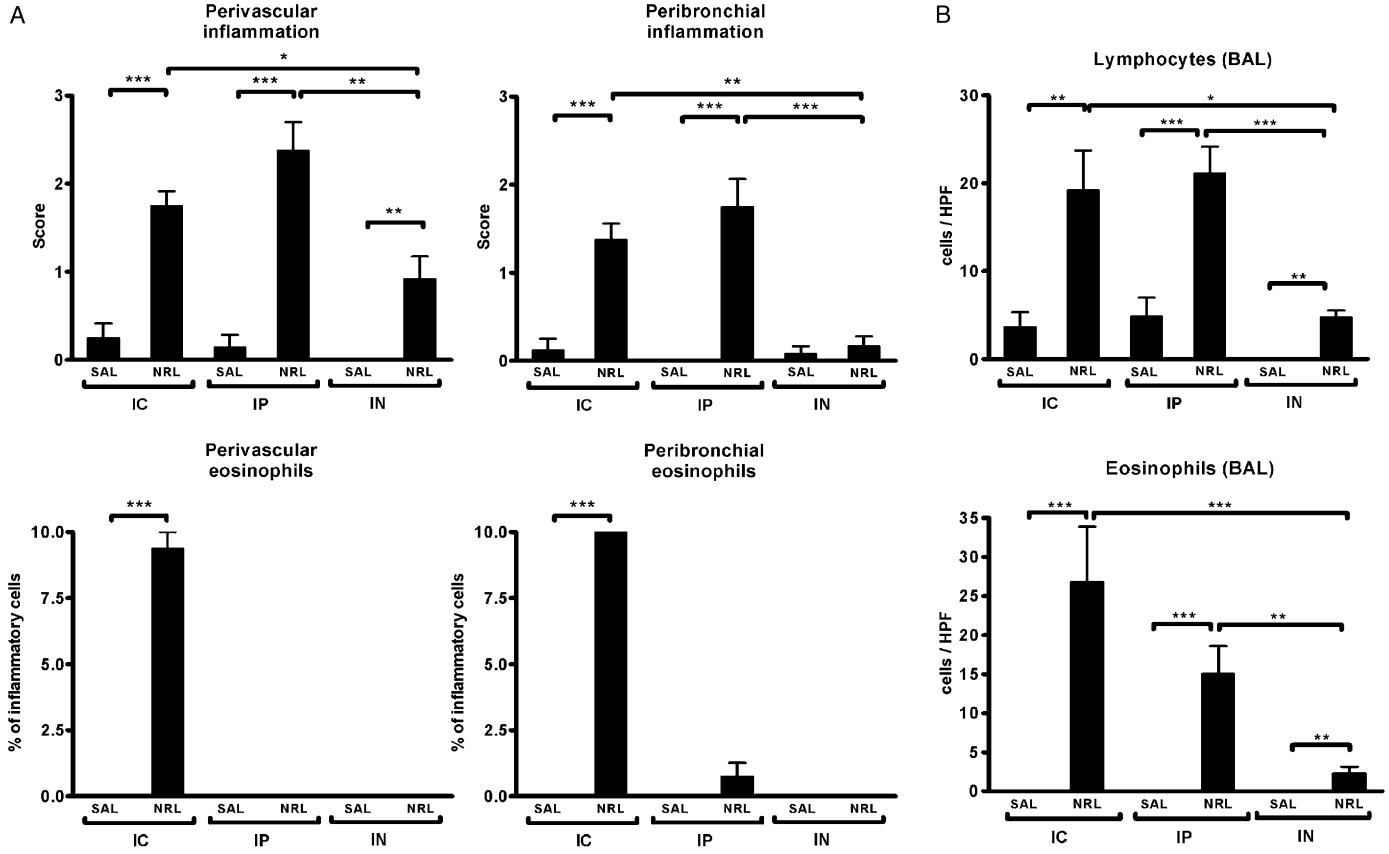

Figure 1
Lung inflammation. (*A*) Inflammation was expressed as score values and eosinophils as percentages of inflammatory cells (n = 7–12 mice per group). (*B*) Lymphocytes and eosinophils in bronchoalveolar lavage (BAL) fluids were counted per high-power field (n = 12–16 mice per group). The columns and error bars represent mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001.

exposed (p < 0.001) but not in intraperitoneally or intranasally exposed mice (Fig 1*A* and S1).

Differential cell counts of bronchoalveolar lavage (BAL) fluid samples indicated a significant eosinophil and lymphocyte recruitment into the lungs of IC and IP NRL-exposed mice as compared with phosphate-buffered saline (PBS)-treated mice (Fig 1*B*). In contrast, eosinophils were virtually absent from the BAL of the mice treated intranasally with NRL, and only a few lymphocytes were detectable (Fig 1*B*).

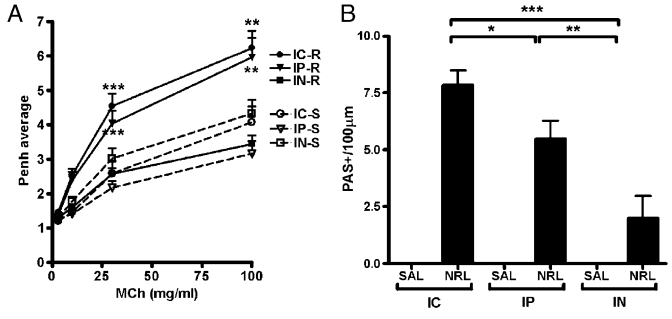

Figure 2
Airway hyperresponsiveness and lung mucus production. (*A*) Airway hyperreactivity was expressed by average enhanced pause (Penh) values in relation to increasing doses of aerosolized methacholine. Data represents mean ± SEM. (*B*) The number of mucus-producing cells was represented as periodic acid-Schiff (PAS)-positive cells. The columns and error bars represent mean ± SEM; ns, non-significant, *p < 0.05, **p < 0.01, ***p < 0.001; n = 12–16 mice per group.

We rarely detected any neutrophils or any significant changes in the number of macrophages in the BAL in any group of mice investigated (data not shown).

IC exposure and challenge with NRL induces marked AHR and lung mucus production AHR to inhaled methacholine (MCh) was analyzed in order to evaluate changes in airway resistance in the response to allergen exposure and challenge. IC and IP NRL exposure followed by airway challenge induced a strong and significant increase in airway reactivity to inhaled MCh (Fig 2*A*). In contrast, IN NRL exposure failed to induce AHR, whereas control mice showed a slightly elevated but statistically non-significant response to inhaled MCh.

Airway mucus overproduction contributes significantly to the pathophysiologic changes in asthma (Maddox and Schwartz, 2002). Lung sections were stained with periodic acid-Schiff (PAS) to quantify the amount of mucus-producing cells around the bronchioles. As shown in Fig 2*B* and S2, animals treated intracutaneously or intraperitoneally with NRL displayed a significant increase in mucus production, as seen in the high amount of goblet cells around the airway lumen. IN NRL exposure, on the other hand, induced only a minor and insignificant increase in mucus production in the airways.

Expression of several CC chemokine mRNA is strongly upregulated in lung tissue after IC and IP exposure to

NRL We analyzed several CC chemokines known to play an important role in allergic airway inflammation (Lukacs, 2001; Bisset and Schmid-Grendelmeier, 2005). Following IC, IP, and also IN NRL exposure and challenge, significant increases in CCL1, CCL8, CCL11, CCL17, and CCL24 mRNA expressions were detected in the lungs relative to PBS-treated mice (Fig 3). The level of CCL3 mRNA was significantly elevated in the lungs after IC and IP NRL exposures, whereas IN exposure did not elicit induction of this chemokine (Fig 3). Expression levels of most of the chemokines were significantly higher after IC latex exposure (CCL1, CCL3, CCL8, CCL11, and CCL24) and IP exposure (CCL1, CCL3, CCL8, CCL17, and CCL24) compared with intranasally exposed mice. CCL3 mRNA levels were significantly higher in intraperitoneally exposed mice compared with intracutaneously exposed mice. In addition, mRNA expression levels of chemokine receptors corresponding to the investigated chemokine ligands are shown in Fig S3.

IC NRL exposure induces strong expression of T-helper type 2 (Th2)-type cytokine mRNA, whereas IN exposure elicits marked induction of transforming growth factor-β1 (TGF-β1) and forkhead box 3 (Foxp3) mRNA in the airways Elevated expression of Th2-type cytokines interleukin (IL)-4, IL-5, and IL-13 in lung tissues is a characteristic feature of the pulmonary allergic response (Oettgen and Geha, 2001). IC and IP exposure to NRL elicited clear increases in the expression levels of IL-4 and IL-13 mRNA, but only IC exposure increased expression of IL-5 mRNA significantly in lung tissue compared with PBS-treated controls (Fig 4*A*). Moreover, an 8-fold enhancement in the level of IL-13 mRNA in IN NRL-exposed mice was observed in comparison with controls. IC exposure elicited significantly higher levels of IL-4, IL-5, and IL-13 mRNA compared with the intranasally exposed group. In addition, IL-4 mRNA levels were significantly elevated in intraperitoneally exposed mice compared with intranasally exposed mice.

A significant enhancement in the mRNA levels of regulatory cytokine IL-10 was observed in all NRL-exposed groups, especially in intranasally treated mice (Fig 4*B*). Interestingly, a significant elevation in the levels of TGF-β1 mRNA was observed after IN and IP NRL exposure ($p < 0.001$ and $p < 0.01$, respectively) but not after IC exposure. The TGF-β1 mRNA levels were significantly higher in IN and IP mice when compared with intracutaneously exposed mice (Fig 4*B*). In addition, Foxp3 transcription factor mRNA expression also enhanced significantly after IP and IN NRL administration but not after IC exposure (Fig 4*B*). Expression increased most significantly after IN latex exposure.

Total and Hev b 6.01-specific immunoglobulin E (IgE) levels are strongly elevated after IC and IP NRL exposure Allergen-specific IgE antibodies are the hallmark of an immediate allergic reaction (Geha *et al*, 2003). Both IC and IP exposure to NRL elicited the strong induction of total IgE antibodies (Fig 5). On the contrary, total serum IgE remained in the baseline level after IN NRL exposure. IC and IP exposure induced high levels of IgE antibodies against major

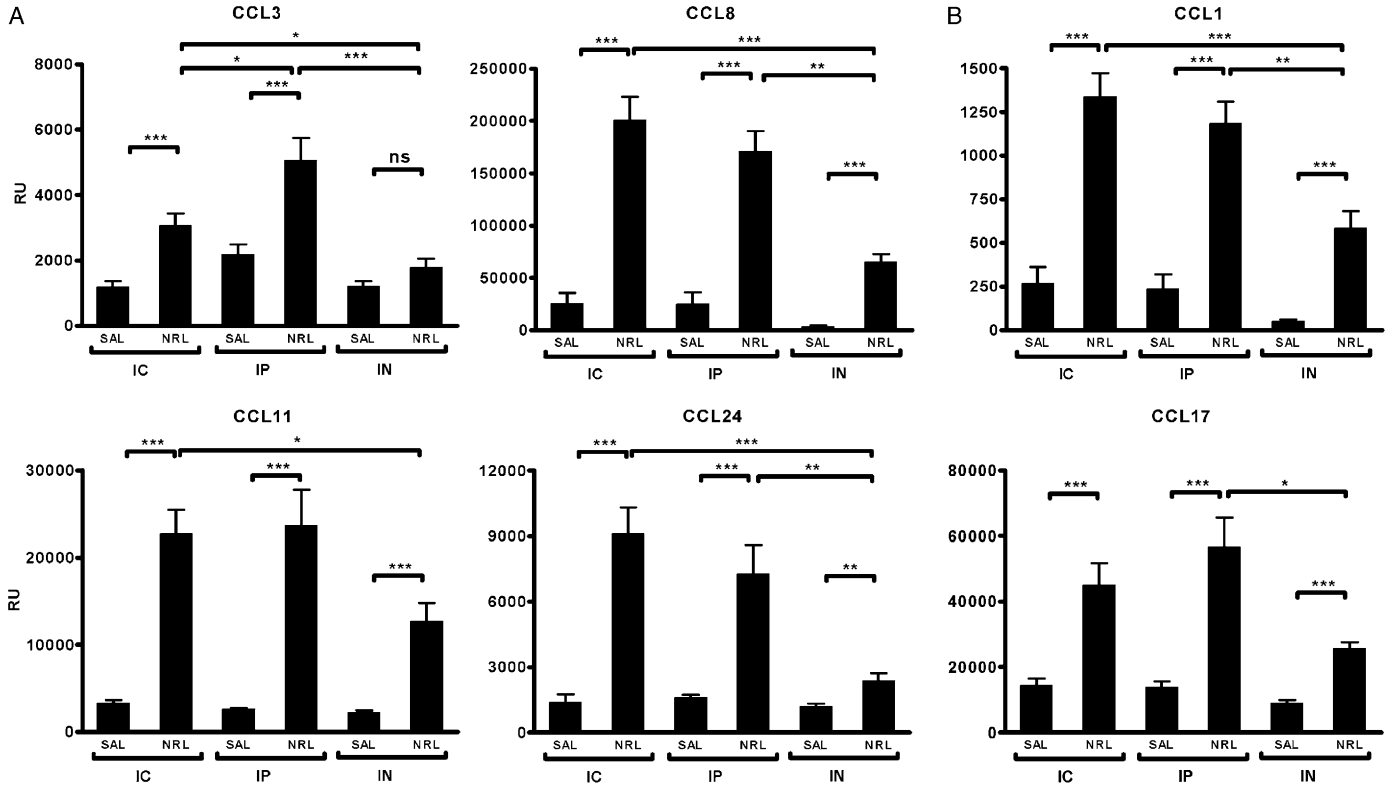

Figure 3
Chemokine mRNA exposure. mRNA expression of chemokines, which attract especially eosinophils (*A*) or T-helper type 2 cells (*B*) in lung samples after exposures to vehicle (saline (SAL)) or natural rubber latex (NRL). Relative units (RU) are relative differences compared with the calibrator. The columns and error bars represent mean ± SEM; ns, non-significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; $n = 12$–15 mice per group.

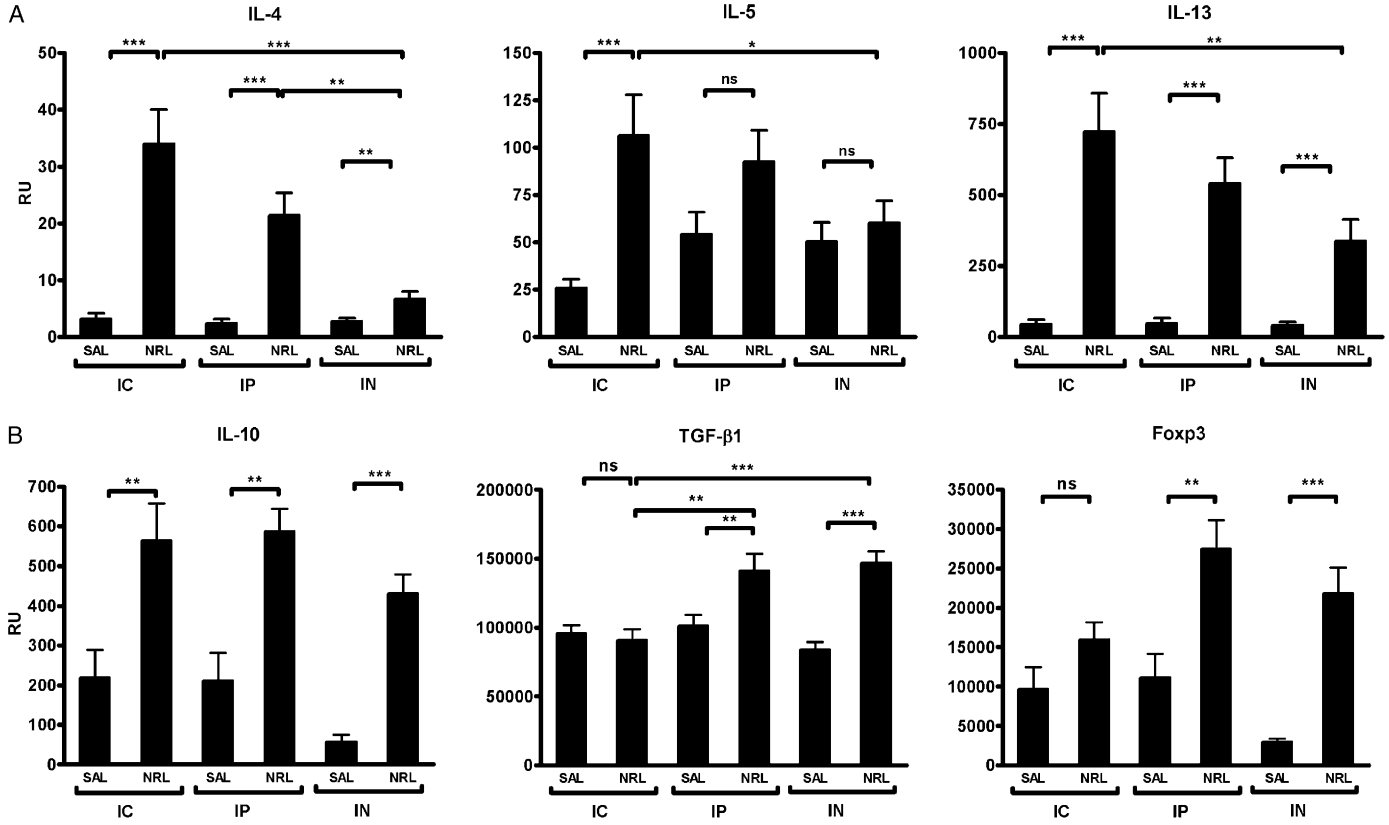

Figure 4
Expression levels of cytokines and Foxp3 mRNAs. T-helper type 2 cytokine mRNA (*A*) and regulatory cytokine and forkhead box 3 (Foxp3) mRNA (*B*) expression in lung samples after exposures to vehicle (saline (SAL)) or natural rubber latex (NRL). The columns and error bars represent mean ± SEM; ns, non-significant, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; n = 12–15 mice per group.

NRL allergen, Hev b 6.01 (Fig 5). In contrast, IN NRL exposure did not induce detectable levels of prohevein (Hev b 6.01)-specific IgE antibodies.

Discussion

Sensitization to NRL products has been one of the leading causes of occupational asthma during the last several years (Fish, 2002). The sensitization routes and pathomechanisms of NRL-induced rhinitis and asthma are, however, not fully understood. As sensitization to NRL proteins may take place through various exposure routes, we investigated their significance in the development of allergic asthma in mouse models. Our findings demonstrate that cutaneous, but not airway, NRL administration in the absence of adjuvant induces a local Th2-dominated lung inflammation and an intense AHR after airway NRL challenge.

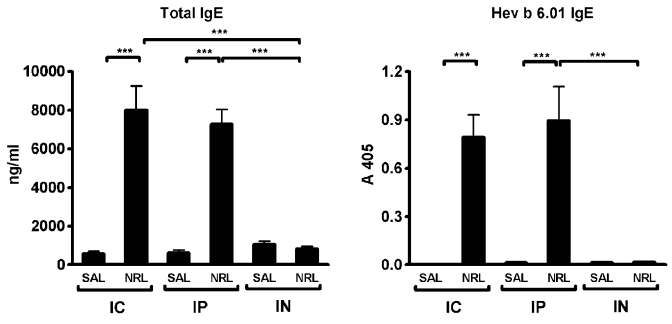

Figure 5
Immunoglobulin E (IgE) levels. Total serum and prohevein (Hev b 6.01)-specific IgE levels in mice after exposures to vehicle (saline (SAL)) or natural rubber latex (NRL) were measured by ELISA. Specific IgE levels of the serum are expressed as absorbance units (405 nm). The columns and error bars represent mean ± SEM; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; n = 12–15 mice per group.

Chronic airway inflammation, an integral feature of allergic asthma, is characterized by the accumulation of inflammatory cells, such as eosinophils (Herrick and Bottomly, 2003). In this study, lung eosinophilia was significantly stronger after IC exposure compared with IN or IP exposures, demonstrating that repeated cutaneous NRL exposure without an external adjuvant is highly efficient in inducing eosinophilic lung inflammation after airway NRL challenge. AHR and airway mucus overproduction contribute significantly to the pathophysiological events in allergic asthma (Herrick and Bottomly, 2003). Both IC and IP exposure, but not IN exposure, followed by NRL airway challenge induced a major increase in AHR to inhaled MCh. Intracutaneously and intraperitoneally exposed mice also showed a significant increase in mucus production. Only a minor increase in mucus production was seen in the airways after IN NRL exposure. In agreement with these findings, Howell *et al* (2002) recently reported that repeated topical NRL exposure to tape-stripped skin elicited an increase in AHR to inhaled MCh, but repeated intratracheal exposure of NRL was unable to induce AHR to inhaled MCh (Howell

et al, 2004). On the other hand, Woolhiser *et al* (2000) previously demonstrated IgE response and AHR following IN exposure of 50 mg of NRL administered 5 d per wk for 10 wk. Our results, however, clearly demonstrate that dermal route exposure using much lower allergen dosage and length of exposure leads to vigorous IgE response, AHR, and airway inflammation after airway challenge.

In order to investigate the mechanisms of leukocyte recruitment to the airways, in detail we studied several CC chemokines in lung tissue. Eosinophils are known to respond to CCL3, CCL5, CCL8, CCL11, and CCL24 (Zimmermann *et al*, 2003). In this study, expressions of CCL8, CCL11, and CCL24 mRNA were significantly higher in intracutaneously exposed mice compared with intranasally exposed mice with marked airway eosinophilia after cutaneous exposure. These chemokines were, however, equally expressed in intraperitoneally and intracutaneously exposed groups although lung eosinophilia was significantly more intense in the intracutaneously exposed mice. Expression levels of CCL1 and CCL17, which are known to attract Th2 cells (Panina-Bordignon *et al*, 2001), were also higher after IC and IP exposure compared with IN exposure. Taken together, our results demonstrate that cutaneous and IP NRL exposure elicits induction of various chemokines in lung tissue, which in turn may recruit Th2-type inflammatory cells, a characteristic of allergic airway inflammation.

A consensus exists that IL-4 and IL-13 are key regulators of IgE class switching (Geha *et al*, 2003). In addition, it has been shown that IL-13 contributes to AHR and mucus overproduction (Kuperman *et al*, 2002). Expression levels of IL-4 and IL-13 mRNA were significantly enhanced after all routes of exposure with NRL in lung tissue in this study. But increases were markedly higher after IC exposure and to a lesser extent after IP exposure, in comparison with IN exposure. The expression level of IL-5, a key cytokine regulating eosinophil recruitment and survival (Hamelmann and Gelfand, 2001), was significantly elevated only after IC NRL exposure. Thus, the greater number of eosinophils in the lungs of intracutaneously exposed mice may be because of an increased eosinophil survival rate induced by increased levels of IL-5 in the airways. On the other hand, CCL24 and IL-5 have been shown to cooperatively promote eosinophil accumulation into airways and to increase AHR to inhaled MCh (Yang *et al*, 2003). Only a few studies exist in the literature describing cytokine expression in lungs after NRL administration. Hardy *et al* (2003) recently reported that mice sensitized intraperitoneally with NRL allergens, and with NRL glove extract demonstrated elevated levels of IL-5 protein in the BAL fluid. They found no significant differences in the levels of IL-4 protein between controls and latex-sensitized groups. Our results clearly indicate that repeated cutaneous NRL exposure induces Th2-dominated cytokine expression in the lungs after airway challenge.

Increased levels of total and allergen-specific IgE antibodies in the patient serum are characteristic of allergic asthma (Busse and Lemanske, 2001; Lemanske and Busse, 2003). In this study, both IC and IP NRL exposure elicited a significant elevation of total serum IgE levels. As an example of allergen-specific IgE response, we investigated antibody responses against Hev b 6.01, which is a major NRL allergen (Alenius *et al*, 1996; Wagner and Breiteneder, 2005). The sera from intracutaneously or intraperitonelly exposed mice contained high levels of IgE antibodies to Hev b 6.01. On the contrary, IN exposure failed to induce elevation of total and specific IgE antibodies. In agreement with these findings, Hufnagl *et al* (2003) also reported increased NRL allergen-specific IgE levels after IP immunization, and a recent study by Woolhiser *et al* (2000) demonstrated that different exposure routes can induce differences in NRL-specific IgE profiles in mice.

It is of interest that airway inflammation, AHR, and IgE levels were substantially lower after IN exposure compared with IC exposure. To address the role of regulatory cytokines in the induction of airway inflammation, we analyzed the mRNA levels of IL-10 and TGF-β1, both of which are known to play important roles in the downregulation of immune responses (Terui *et al*, 2001; Akbari *et al*, 2003; Nagler-Anderson *et al*, 2004). Proportional increase (allergen-exposed mice *vs* PBS-exposed control) in the expression of IL-10 mRNA was markedly more prominent in intranasally exposed mice (7.4-fold increase) compared with intracutaneously (2.6-fold increase) and intraperitoneally exposed (2.8-fold increase) mice. In line with these results, Akbari *et al* (2001) have reported that respiratory exposure to allergens can induce T cell tolerance, which appears to be mediated by IL-10 production. On the other hand, we found significant induction of TGF-β1 mRNA only after IN and IP NRL exposure but not after IC exposure. Furthermore, proportional expression of transcription factor Foxp3, which is expressed predominantly by CD4 + CD25 + T regulatory cells (Fontenot *et al*, 2003; Khattri *et al*, 2003), was significantly upregulated after IN exposure (7.3-fold) but to a lesser extent after IP exposure (2.5-fold) and IC exposure (1.6-fold). Thus, it is possible that increased levels of IL-10 and TGF-β1 as well as Foxp3 + regulatory T cells in the lungs of intranasally exposed mice suppress the inflammatory responses, resulting in diminished airway inflammation. The link between TGF-β and regulatory T cells is supported by the finding that TGF-β is able to promote differentiation of T cells into Foxp3 + regulatory T cells (Chen *et al*, 2003; Fantini *et al*, 2004).

These findings underline the role of cutaneous route allergen exposure in the elicitation of allergic airway inflammation and AHR. Instead of epicutaneous (Spergel *et al*, 1998; Lehto *et al*, 2003) allergen application, we used IC allergen exposure assuring that the actual allergen dose entering the body was the same in all exposure routes. The present observations are of importance when considering prevention of airway hypersensitivity to NRL allergens. Glove powder is the most important carrier of NRL allergens to airways, and thus primary prevention has mainly focused on the use of non-powdered latex gloves (Charous *et al*, 2002). As the exposure via the cutaneous route could be an important way for sensitizing airways, attention will need to be paid to the use of low- or non-allergen NRL gloves instead of using only non-powdered gloves.

Materials and Methods

Allergens NRL and Hev b 6.01 were purified as earlier described (Lehto *et al*, 2003).

Mice exposure protocols and AHR Six- to eight-week-old female BALB/cJBom mice were obtained from Taconic M&B A/S (Ry, Denmark), Social and Health Services of Finland; Provincial Office of Southern Finland approved all experimental protocols.

Exposure protocols are shown in Fig 6. Mice were exposed for 4 wk before airway challenge. In saline (SAL) groups exposures were made with PBS and with NRL in NRL groups. Mice were anesthetized with isoflurane (Abbott Laboratories Ltd, Queenborough, UK) for IC and IN exposure. The backs of the mice were shaved and tape-stripped (Tegaderm, 3M Health Care, St Paul, Minnesota) four times before IC exposure to remove loose hair and to induce standardized skin injury mimicking the scratching that is a characteristic feature in patients with atopic dermatitis. In IC exposure, mice were injected once a week with 40 μg of NRL in 100 μL of PBS or with 100 μL PBS as control. IN exposure was also made once a week with the same amounts of NRL in 50 μL PBS. In IP exposure, mice were injected on days 1 and 14 with 80 μg NRL together with 2 mg alum (Pierce, Rockford, Illinois) in 100 μL PBS. Mice were challenged after exposures via the respiratory route using an ultrasonic nebulizator (Aerogen Ltd, Galway, Ireland) with 0.5% NRL (in PBS) for 20 min on days 28, 29, and 30. On day 31, AHR to MCh (Sigma-Aldrich Co, St Louis, Missouri) was measured by using whole-body plethysmography (Buxco Electronics Inc., Sharon, Connecticut) as previously described (Hamelmann *et al*, 1997) and monitored by average Penh (enhanced pause = pause × (peak inspiratory box flow/peak expiratory box flow)) values. MCh concentrations were 3, 10, 30, and 100 mg per mL. The mice were then sacrificed with carbon dioxide, and samples (BAL fluids, lung biopsies, and blood) were taken for subsequent analysis. The experiment was repeated two times, and the combined results are presented.

BAL and lung histology Lungs were lavaged with 0.8 mL of PBS, and BAL specimens were cytocentrifuged (Shandon Scientific Ltd, Runcorn, UK) at 150 × *g* for 5 min. Cells were stained with May–Grünwald–Giemsa and counted blinded in 15–20 high-power fields at × 1000 under light microscopy.

Formalin-fixed lungs were embedded in paraffin, and lung sections (4 μm) were stained with H&E or with PAS solution. Inflammation in different lung areas was expressed after H&E staining as score values 1–5 (1, slightly inflamed tissue; 2, inflammation of the area over 20%; 3, inflammation of the area over 50%; 4, inflammation of the area over 75%; and 5, entire tissue inflamed). The number of mucus-producing cells was determined after PAS staining by choosing three bronchial sections randomly and counting PAS-positive cells per 100 μm. The slides were analyzed blinded with a Nikon EclipseE800 microscope (Leica Microsystems GmbH, Wetzlar, Germany).

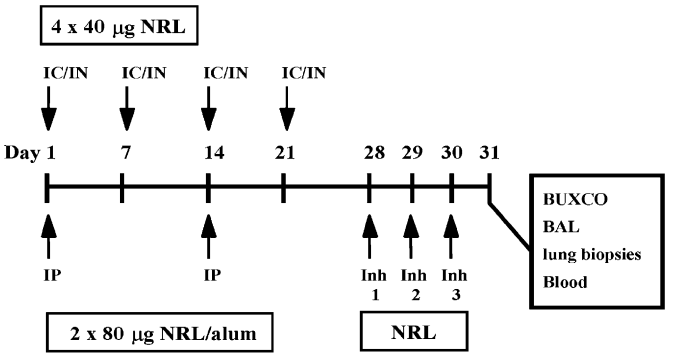

Figure 6
Intracutaneous (IC), intraperitoneal (IP), and intranasal (IN) exposure schedules. Mice were exposed for 4 wk before airway challenge. In saline (SAL) groups exposures were made with phosphate-buffered saline and with natural rubber latex (NRL) in NRL groups. Airway challenges were made by NRL. Airway hyperreactivity to methacholine was measured 24 h after airway challenges and different samples were taken for subsequent analysis.

Total and specific IgE antibodies in serum Total IgE levels were measured as previously described (Lehto *et al*, 2003). A specific ELISA for purified Hev b 6.01 allergen was slightly modified. Plates were first coated for 3 h at +20°C and then overnight at +4°C. The blocking time was 1 h at +20°C and 1:10 diluted samples were incubated for 2 h at +20°C. Biotin-labeled anti-mouse isotype-specific antibody was added for 2 h at +20°C. Streptavidin-conjugated alkaline phosphatase (Zymed, San Francisco, California, diluted 1:1000) was incubated for 30 min at +20°C, color substrate (*p*-nitrophenyl phosphate, Sigma-Aldrich Co) was added, and absorption at 405 nm was read. The sera, biotinylated anti-mouse antibodies, and streptavidin-conjugated phosphatase were diluted in 0.2% BSA/0.05% Tween/PBS.

Expression of mRNA in lungs RNA extraction and synthesis of cDNA was carried out as described earlier (Lehto *et al*, 2003). Real-time quantitative PCR was performed with an AbiPrism 7700 Sequence Detector System (SDS) (Applied Biosystems, Foster City, California) as previously described (Lehto *et al*, 2003). PCR primers and probes were from Applied Biosystems as predeveloped reagents (18S ribosomal RNA, IL-4, IL-5, IL-10, IL-13, CCL1, CCL3, and Foxp3) or were self-designed. The self-designed sequences of CCL11, CCL17, and CCL24 have been described earlier (Lehto *et al*, 2003), and others are as follows: TGF-β1 (forward 5′-CAA GGG CTA CCA TGC CAA CTT-3′, probe 5′-CAC ACA GTA CAG CAA GGT CCT TGC CCT CT-3′, and reverse 5′-ATG GGC AGT GGC TCC AAA G-3′), CCL8 (forward 5′-CCC TTC GGG TGC TGC AAA G-3′, probe 5′-TAC GAG AGA ATC AAC AAT ATC CAG TGC CCC-3′, and reverse 5′-TCT GGA AAA CCA CAG CTT CCA-3′). The results are expressed as relative units (RU), which were calculated by the comparative C_T method (Lehto *et al*, 2003).

Statistics Statistical tests were performed using GraphPad Prism Version 4 (GraphPad Software Inc.). Single group comparisons were conducted by the non-parametric Mann–Whitney *U* test. A p-value of less than 0.05 was considered to be statistically significant.

This study was supported by grants from the Academy of Finland (grants number 37852 and 50809). The Ansell Healthcare Corporation also funded this work. We thank Ms Helena Honkasalo and Ms Pirjo Tuomi for their expert and excellent technical assistance.

Supplementary Material

The following material is available online for this article.
Figure S1 Histology of H & E stained penvascular using tissues.
Figure S2 Histology of PAS-stained peribronchial lung sections.
Figure S3 Chemokine receptor mRNA expression.

DOI: 10.1111/j.0022-202X.2005.23910.x

Manuscript received April 8, 2005; revised June 6, 2005; accepted for publication June 21, 2005

Address correspondence to: Harri Alenius, PhD, Laboratory of Immunotoxicology, Finnish Institute of Occupational Health, Topeliuksenkatu 41 aA, FIN-00250 Helsinki, Finland. Email: Harri.Alenius@ttl.fi

References

Ahmed DD, Sobczak SC, Yunginger JW: Occupational allergies caused by latex. Immunol Allergy Clin North Am 23:205–219, 2003

Akbari O, DeKruyff RH, Umetsu DT: Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. Nat Immunol 2:725–731, 2001

Akbari O, Stock P, DeKruyff RH, Umetsu DT: Role of regulatory T cells in allergy and asthma. Curr Opin Immunol 15:627–633, 2003

Alenius H, Kalkkinen N, Reunala T, Turjanmaa K, Palosuo T: The main IgE-binding epitope of a major latex allergen, prohevein, is present in its N-terminal 43-amino acid fragment, hevein. J Immunol 156:1618–1625, 1996

Bernstein DI, Biagini RE, Karnani R, *et al*: *In vivo* sensitization to purified *Hevea brasiliensis* proteins in health care workers sensitized to natural rubber latex. J Allergy Clin Immunol 111:610–616, 2003

Bernstein DI, Karnani R, Biagini RE, *et al*: Clinical and occupational outcomes in health care workers with natural rubber latex allergy. Ann Allergy Asthma Immunol 90:209–213, 2003

Bisset LR, Schmid-Grendelmeier P: Chemokines and their receptors in the pathogenesis of allergic asthma: Progress and perspective. Curr Opin Pulm Med 11:35–42, 2005

Busse WW, Lemanske RF Jr: Asthma. N Engl J Med 344:350–362, 2001

Charous BL, Blanco C, Tarlo S, *et al*: Natural rubber latex allergy after 12 y: Recommendations and perspectives. J Allergy Clin Immunol 109:31–34, 2002

Chen W, Jin W, Hardegen N, *et al*: Conversion of peripheral CD4 + CD25 − naive T cells to CD4 + CD25 + regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med 198:1875–1886, 2003

Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF: Cutting edge: TGF-beta induces a regulatory phenotype in CD4 + CD25 − T cells through Foxp3 induction and down-regulation of Smad7. J Immunol 172: 5149–5153, 2004

Fish JE: Occupational asthma and rhinoconjunctivitis induced by natural rubber latex exposure. J Allergy Clin Immunol 110:S75–S81, 2002

Fontenot JD, Gavin MA, Rudensky AY: Foxp3 programs the development and function of CD4 + CD25 + regulatory T cells. Nat Immunol 4:330–336, 2003

Geha RS, Jabara HH, Brodeur SR: The regulation of immunoglobulin E class-switch recombination. Nat Rev Immunol 3:721–732, 2003

Hamelmann E, Gelfand EW: IL-5-induced airway eosinophilia—the key to asthma? Immunol Rev 179:182–191, 2001

Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand EW: Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. Am J Respir Crit Care Med 156: 766–775, 1997

Hardy CL, Kenins L, Drew AC, Rolland JM, O'Hehir RE: Characterization of a mouse model of allergy to a major occupational latex glove allergen Hev b 5. Am J Respir Crit Care Med 167:1393–1399, 2003

Herrick CA, Bottomly K: To respond or not to respond: T cells in allergic asthma. Nat Rev Immunol 3:405–412, 2003

Howell MD, Tomazic VJ, Leakakos T, Truscott W, Meade BJ: Immunomodulatory effect of endotoxin on the development of latex allergy. J Allergy Clin Immunol 113:916–924, 2004

Howell MD, Weissman DN, Jean Meade B: Latex sensitization by dermal exposure can lead to airway hyperreactivity. Int Arch Allergy Immunol 128: 204–211, 2002

Hufnagl K, Wagner B, Winkler B, *et al*: Induction of mucosal tolerance with recombinant Hev b 1 and recombinant Hev b 3 for prevention of latex allergy in BALB/c mice. Clin Exp Immunol 133:170–176, 2003

Khattri R, Cox T, Yasayko SA, Ramsdell F: An essential role for Scurfin in CD4 + CD25 + T regulatory cells. Nat Immunol 4:337–342, 2003

Kuperman DA, Huang X, Koth LL, *et al*: Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. Nat Med 8:885–889, 2002

Lehto M, Koivuluhta M, Wang G, *et al*: Epicutaneous natural rubber latex sensitization induces T helper 2-type dermatitis and strong proheveinspecific IgE response. J Invest Dermatol 120:633–640, 2003

Lemanske RF Jr, Busse WW: 6. Asthma. J Allergy Clin Immunol 111:S502–S519, 2003

Lukacs NW: Role of chemokines in the pathogenesis of asthma. Nat Rev Immunol 1:108–116, 2001

Maddox L, Schwartz DA: The pathophysiology of asthma. Annu Rev Med 53:477–498, 2002

Nagler-Anderson C, Bhan AK, Podolsky DK, Terhorst C: Control freaks: Immune regulatory cells. Nat Immunol 5:119–122, 2004

Nolte H, Babakhin A, Babanin A, *et al*: Prevalence of skin test reactions to natural rubber latex in hospital personnel in Russia and eastern Europe. Ann Allergy Asthma Immunol 89:452–456, 2002

Oettgen HC, Geha RS: IgE regulation and roles in asthma pathogenesis. J Allergy Clin Immunol 107:429–440, 2001

Panina-Bordignon P, Papi A, Mariani M, *et al*: The C–C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. J Clin Invest 107:1357–1364, 2001

Reunala T, Alenius H, Turjanmaa K, Palosuo T: Latex allergy and skin. Curr Opin Allergy Clin Immunol 4:397–401, 2004

Sparta G, Kemper MJ, Gerber AC, Goetschel P, Neuhaus TJ: Latex allergy in children with urological malformation and chronic renal failure. J Urol 171:1647–1649, 2004

Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS: Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. J Clin Invest 101:1614–1622, 1998

Terui T, Sano K, Shirota H, *et al*: TGF-beta-producing CD4 + mediastinal lymph node cells obtained from mice tracheally tolerized to ovalbumin (OVA) suppress both Th1- and Th2-induced cutaneous inflammatory responses to OVA by different mechanisms. J Immunol 167:3661–3667, 2001

Turjanmaa K, Makinen-Kiljunen S: Latex allergy: Prevalence, risk factors, and cross-reactivity. Methods 27:10–14, 2002

Wagner S, Breiteneder H: *Hevea brasiliensis* latex allergens: Current panel and clinical relevance. Int Arch Allergy Immunol 136:90–97, 2005

Weissman DN, Lewis DM: Allergic and latex-specific sensitization: Route, frequency, and amount of exposure that are required to initiate IgE production. J Allergy Clin Immunol 110:S57–S63, 2002

Woolhiser MR, Munson AE, Meade BJ: Immunological responses of mice following administration of natural rubber latex proteins by different routes of exposure. Toxicol Sci 55:343–351, 2000

Yang M, Hogan SP, Mahalingam S, *et al*: Eotaxin-2 and IL-5 cooperate in the lung to regulate IL-13 production and airway eosinophilia and hyperreactivity. J Allergy Clin Immunol 112:935–943, 2003

Zimmermann N, Hershey GK, Foster PS, Rothenberg ME: Chemokines in asthma: Cooperative interaction between chemokines and IL-13. J Allergy Immunol Clin 111:227–242; 2003 quiz 243