IL-24 contributes to skin inflammation in Para-Phenylenediamine-induced contact hypersensitivity

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Para-Phenylenediamine (PPD) is an aromatic amine used in hair dyes and in temporary black henna tattoos, which is a frequent cause of allergic contact dermatitis (ACD). ACD is a skin inflammatory reaction characterized by modifications such as spongiosis, exocytosis and acanthosis. The aim of this study is to characterize the expression and the role of IL-20-related cytokines, including IL-19, IL-20, IL-22 and IL-24, in ACD. The expression of IL19, IL20, IL22 and IL24 is increased in affected skin from PPD allergic patients compared with uninvolved skin. In addition, the expression of these cytokines positively correlates with clinical symptoms. To assess their role in ACD, we set up a mouse model of PPD-induced allergic contact dermatitis and we showed that, in contrast to Il22-deficient mice, Il20rb- and Il24-deficient mice are partially protected against development of PPD-induced contact hypersensitivity. These mice have decreased ear thickening and less acanthosis compared with WT mice after PPD treatment. In addition, the absence of IL-22R, IL-20R2 or IL-24 affects the recruitment of neutrophils into the skin but not the total IgE production. Taken together, these results demonstrate the implication of IL-24 via the IL-20R type II receptor in the inflammatory process of ACD.

Para-Phenylenediamine (PPD) is an aromatic amine used in hair dyes and in temporary black henna tattoos1. Because of its potent allergenic properties, hairdressers or consumers of hair dye products can develop allergic contact dermatitis (ACD). ACD is a skin inflammatory reaction characterized by modifications such as spongiosis, exocytosis and acanthosis. The aim of this study is to characterize the expression and the role of IL-20-related cytokines, including IL-19, IL-20, IL-22 and IL-24, in ACD. The expression of IL19, IL20, IL22 and IL24 is increased in affected skin from PPD allergic patients compared with uninvolved skin. In addition, the expression of these cytokines positively correlates with clinical symptoms. To assess their role in ACD, we set up a mouse model of PPD-induced allergic contact dermatitis and we showed that, in contrast to Il22-deficient mice, Il20rb- and Il24-deficient mice are partially protected against development of PPD-induced contact hypersensitivity. These mice have decreased ear thickening and less acanthosis compared with WT mice after PPD treatment. In addition, the absence of IL-22R, IL-20R2 or IL-24 affects the recruitment of neutrophils into the skin but not the total IgE production. Taken together, these results demonstrate the implication of IL-24 via the IL-20R type II receptor in the inflammatory process of ACD.
treatment by corticosteroid is necessary. A better understanding of PPD allergy mechanisms seems to be essential to improve prevention and treatment.

Because IL-20-related cytokines are known to play an important role in skin inflammatory diseases such as psoriasis, they could be actors in the ACD reaction. IL-20-related cytokines are produced by immune cells such as monocytes and T lymphocytes and are involved in the maintenance of the epidermal barrier integrity by promoting antimicrobial peptide production, chemokine expression and keratinocyte proliferation. These cytokines play redundant roles because they share common receptor complexes. IL-19, IL-20 and IL-24 can bind to the "type I IL-20 receptor" composed of IL-20R1 and IL-20R2. The "type II IL-20 receptor" consists of IL-22R and IL-20R2 and binds IL-20 and IL-24. Finally, IL-22 signals through a complex composed of an IL-22R subunit and IL-10R2.

Even if the biological activities of IL-20-related cytokines are beneficial during wound healing or pathogen invasion, these cytokines might play a detrimental role in inflammatory skin disorders. For instance, they are upregulated in skin lesions from psoriatic patients and we showed that IL-22 is implicated in keratinocyte proliferation and abnormal differentiation as well as in neutrophil infiltration in a mouse model of psoriasis. In addition, transgenic mice for IL-20, IL-22 and IL-24 but not IL-19 display a thickened skin due to acanthosis, demonstrating the role of IL-22R-binding cytokines in this skin inflammatory disorder. In ACD, very little is known about the role of IL-20-related cytokines. IL-22 is found in the serum of nickel-allergic patients and is produced by CD4+ T lymphocytes that are present in the skin of these patients. An increase of IL22 expression in the skin has also been reported in murine contact hypersensitivity (CHS) models induced by TNCB or oxazolone. In addition, the expression of IL19 and IL24 is also increased in a mouse model of CHS induced by DNFB. In another model of TNCB-induced CHS, it was shown that IL20rb-deficient mice were more affected than WT mice, suggesting that IL-19, IL-20 and IL-24 play a protective role.

In PPD-induced contact dermatitis, no information is available about the expression of IL-20-related cytokines in the skin of patients, nor in animal models. Here, we show that the expression of IL19, IL22 and IL24 was increased in both human and mouse models of PPD-induced CHS. In addition, IL22ra-, IL20rb- and IL24-deficient mice were partially protected against development of CHS, demonstrating a detrimental role of IL-24 via its effect on type II IL-20R in PPD-induced CHS.

**Results**

**IL-20 subfamily cytokines are expressed in the skin of PPD-allergic patients.** We evaluated the expression of IL-20 subfamily cytokines in patients diagnosed for PPD-induced ACD. Before patch testing with the commercial allergen PPD, skin biopsies of uninvolved skin were collected (mentioned as PPD 0 hour). 8 hours, 24 hours and 48 hours after PPD application, allergic reactions were evaluated and skin biopsies were performed. 7 out of 11 patients presented a positive patch test after 24 hours, 3 patients only after 48 hours and for one patient tests remained negative even after 72 hours (Suppl. Table 1). As shown in Fig. 1, the expression of IL19, IL20, IL22 and IL24 was increased after PPD patch application and expression correlated with clinical observations. The increased expression of these cytokines in biopsies was less pronounced and clearly delayed in patients who showed no clinical signs at 24 hours (grey curves in Fig. 1A) compared with the 7 most rapidly affected patients (black curves in Fig. 1A). IL24 was also induced, upon in vitro stimulation, in PBMCs of allergic patients compared to PBMCs of healthy controls. In contrast, the expression of IL19 and IL22 was similar in PBMCs of healthy controls compared to PBMCs of allergic patients, where IL20 was not detectable (Fig. 1B). Altogether these data suggest that the IL-20-related cytokines might play a role in PPD-induced ACD. To examine the role of these cytokines in ACD, we developed a mouse model adapted from two other models. Mitsoura et al. demonstrated that the IL-20-related cytokines might play a role in PPD-induced ACD. To examine the role of these cytokines in ACD, we developed a mouse model adapted from two other models.

**Development of a mouse model of PPD-induced ACD.** Mice treated with PPD showed a significant ear thickening starting after the third PPD application (Suppl. Fig. 1A). As observed in Suppl. Fig. 1A, mice that did not undergo the two first applications did not develop any ear thickening, indicating that preliminary sensitization is essential for PPD-induced CHS development. In addition, Rag2−/− mice did not respond to PPD treatment in contrast to WT mice (Suppl. Fig. 1B). We also observed an increase in IgE level in sera from mice treated with PPD compared with vehicle control mice (VC), demonstrating the presence of an allergic reaction in our model (Suppl. Fig. 1C). At histological level, PPD-treated ear sections displayed the distinctive features of human ACD such as edema, neutrophilic infiltrate, dermal inflammatory infiltrate, exocytosis and acanthosis (Suppl. Fig. 1D). Moreover, in contrast to vehicle control mice, mice treated with PPD showed an infiltration of TCR β+ cells in the dermis and epidermis, as in human ACD (Suppl. Fig. 1E).

**Upregulation of the expression of IL-20-related cytokines upon PPD treatment.** First, expression of IL-20-related cytokines was examined in this model on the total skin. During the early phase (24 hours after the second application), we observed an induction of IL19, IL22 and IL24 mRNA expression after PPD application compared with control skin, whereas IL20 expression was decreased by PPD treatment (Fig. 2A). Expression of IL19 and IL22 was also induced during the late phase (24 hours after the third application) in contrast to IL20 expression or IL22 expression, which is not detected (Suppl. Fig. 2A). To determine whether hematopoietic cells or keratinocytes represent the main source of these cytokines, we purified CD45+ and CD45− cells from the epidermis (Suppl. Fig. 2B). As expected, Cd3ε expression was restricted to the CD45-positive fraction and increased after PPD challenges, reflecting the T cell infiltration observed by flow cytometry (Suppl. Fig. 2C). Krt10, a keratinocyte marker, was mainly expressed in the CD45-negative fraction, confirming enrichment of keratinocytes in this fraction (Suppl. Fig. 2C). Interestingly, IL19 and IL24 expression appeared to be upregulated by PPD treatment in both fractions, although statistical significance was reached only in the CD45-negative fraction (Fig. 2B). IL20 was not significantly affected and IL22 expression was only upregulated in CD45-positive cells, at a later time point (at day 12, after five PPD applications) (Fig. 2B).
Taken together these results indicate that expression of IL19, IL22 and IL24 are quickly upregulated after PPD treatment, and non-hematopoietic cells represent the main source of IL-19 and IL-24 whereas CD45+ cells produce IL-22.

**II22ra1-, II20rb and II24-deficient mice are partially protected against PPD-induced CHS.** To analyze the role of these IL-20 subfamily cytokines in CHS, we treated II22-, II24-, II22ra1- and II20rb-deficient mice with PPD. II22-deficient mice showed a similar ear thickening as WT mice (Fig. 3A). In contrast, II22ra1-deficient mice showed a partial protection against the development of CHS displaying less ear swelling compared with PPD-treated WT littermates (Fig. 3B), as well as less acanthosis (Fig. 4A,B). This observation suggested that IL-22R plays a role in this model, independently of IL-22 activity. As II24 expression is strongly increased upon PPD treatment and IL-22R can associate with IL-20R2 to form a receptor complex for IL-24, we hypothesized that II24- and II20rb-deficient mice might be protected in the same way as II22ra1-deficient mice. As shown in Fig. 3C and D, II24- and II20rb-deficient mice showed a partial protection, which is associated with less acanthosis (Fig. 4C-F) and a smaller proportion of crusts (Fig. 4C-F) compared to WT littermate mice.

The absence of IL-24 activity does not affect the IgE-dependent allergy but partially prevents the epidermal infiltration of CD45+ cells and neutrophils induced by PPD. To analyze the allergic process, we measured the IgE levels in the sera of II20rb-, II24-, II22ra1-deficient mice. We showed similar IgE level than WT mice (Fig. 5), suggesting that the IgE-dependent allergy was still present in deficient mice. We then examined the immune cells infiltrate in the ear skin and we observed that the proportion of CD45+ cells was increased after PPD treatment in the epidermis of WT mice and to a lesser extent in II20rb- and II24- deficient mice (Fig. 6A). We observed similar results in the dermis (Suppl. Fig. 3A). As TCRβ+ cells are known to play a role in CHS, we studied the proportion of TCRβ+ cells after PPD application. The percentage of TCRβ+ cells was increased in the dermis and epidermis of PPD-treated mice but no difference was observed between WT and II20rb-, II24-, II22ra1-deficient mice (Fig. 6B and Suppl. Fig. 3B). Another population present at a high percentage (20–30%) after PPD treatment is Ly6G+CD11B+ cells, which are neutrophils (Fig. 6C). In both epidermis and dermis, the percentage of Ly6G+CD11B+ was significantly lower in II20rb-, II24-, II22ra1-deficient mice.
mice compared to WT mice (Fig. 6C and Suppl. Fig. 3C). We hypothesized that this difference in neutrophil infiltrate could explain the protective effect observed in deficient mice. We did not observe any difference in neutrophil activity based on CD11B staining between neutrophils from WT versus Il22ra1-deficient mice (Fig. 7A,B).

However, when we induced neutrophil recruitment in ears by injecting a cocktail of Cxcl1 and Ccl3, two chemokines known to be induced by IL-22R and able to induce neutrophil recruitment, we observed a significant increase in ear thickening (Fig. 7C). We confirmed that ear thickness was correlated with the percentage of neutrophils in the epidermis, because this percentage was higher after chemokine injection (Fig. 7D). In addition, we noticed that the significant decrease in the immune cell infiltrate in deficient mice happened during the early phase of PPD treatment but not during the late phase (Suppl. Fig. 4), suggesting that IL-24 is involved in the early phase of the inflammatory reaction. Moreover, in auricular lymph nodes, we did not observe any difference in cell number, cytokine expression (Il4, Ifng and Il17) and cell composition (B220, CD3, CD4 and CD8) in Il20rb-, Il24- and Il22ra1-deficient mice compared to WT mice (Suppl. Figs 5 and 6). These data suggest that protection in the absence of IL-24 occurs mainly in the skin and is not associated with global inflammation. Together, these observations indicate that IL-24 plays a role in the local, early inflammatory reaction induced by PPD application. It induces acanthosis and increases the inflammatory infiltrate, particularly neutrophils, without affecting IgE-dependent allergy.

Discussion

Our results indicate that the IL-20-related cytokines are markers of PPD allergy because we found a correlation between IL-20-related cytokine expression and the severity of reactions in patients. We also demonstrated that IL-20-related cytokines, and more particularly IL-24, play a role in PPD-induced CHS since Il24-, Il22ra1- and Il20rb-deficient mice were partially protected against the development of acanthosis and the neutrophil influx in our mouse model.

Here, we have developed a mouse model that recapitulates the typical features of PPD-induced ACD with respect to spongiosis, exocytosis and inflammatory infiltrate. As expected in murine CHS model, the sensitization phase takes 5–7 days whereas in human it takes 10–15 days. After 6 days, we already observed a massive infiltrate of αβ T cells in PPD-treated skin in contrast to other skin diseases, such as the imiquimod-induced
psoriasis model where γδ T cells play an important role. Our data indicate that the PPD-induced CHS model is associated with a Th2 response because mice treated with PPD have an increased expression of Il4 in the skin (data not shown) and a high IgE blood level. In addition, we observed an important induction of Il19 and Il24 expression, which are both Th2-associated cytokines. Different studies have already shown that repeated elicitation is associated with a Th2 response, while acute ACD is rather Th1-dominated. In contrast, we observed no Il17 expression (data not shown) after PPD application suggesting that Th17 cells do not play a major role in our CHS model, while IL-17 plays an important role in DNFB and TNCB-induced CHS. High levels of Th17 cytokines were also detected in the blood and in the skin of patients suffering from nickel-induced contact dermatitis demonstrating the association of Th17 and ACD with some allergens.

Il24−/−, Il22ra1−/− and Il20rb−/− mice are partially protected against PPD-induced CHS, as shown in Figure 3. Ear thickness was measured before each PPD treatment and 24 hours after the last application with a micrometer screw to evaluate the development of CHS. WT mice showed a significant increase in ear thickness compared to VC mice (two-way ANOVA, Bonferroni multiple comparison).

Our results demonstrate that IL-24, but not IL-22, is required for the skin reaction induced by PPD application. However, we cannot exclude that IL-19 and IL-20, which act via IL-20R2 and are highly expressed after PPD application in allergic patients, could also play a role in the development of CHS. Nevertheless, Il20rb−/− deficient mice showed the same level of protection as Il22ra1−/− mice, suggesting that IL-19 does not play a major role in this model. In line with this result, a study demonstrated that IL-19 blood level correlates with disease severity in psoriasis but IL-19 alone has only few effects on keratinocyte proliferation and migration. Instead, IL-19 strengthens the action of IL-17A by amplifying the expression of antimicrobial peptides and neutrophil-attracting chemokines. As IL-17 is not produced in our model of PPD-induced CHS, the role of IL-19 is unlikely. In contrast, based on the more prominent protection in Il20rb−/− mice compared to Il24−/− mice, IL-20, which is constitutively expressed in our model, could indeed play a role. Of note, IL-20 has proinflammatory roles in autoimmune diseases such as psoriasis and rheumatoid arthritis. IL-20 induces epidermal hyperplasia and inhibits terminal keratinocyte differentiation in human epidermis. IL-20 also induces the production of proinflammatory cytokines, including TNF-α and IL-1β, by synovial fibroblasts.

Figure 3.
Neutrophil influxes were lower in the epidermis and dermis of Il24−/−, Il22ra1−/− and Il20rb−/− mice compared to WT littermates, in line with lower levels of chemokine associated with neutrophil recruitment such as Cxcl3, Ccl3 or Cxcl5 (data not shown). We confirmed the role of neutrophils in ear thickening in our model by injecting Cxcl1 and Ccl3 chemokines in mice ears. The role of neutrophils was also highlighted in a study that reported the importance of neutrophils during both sensitization and elicitation phases of CHS42. Neutrophils are required for the CHS response because absence of neutrophils during sensitization phase abrogates ear thickness and inflammatory response42.

Figure 4. Acanthosis is less prominent in Il24−/−, Il22ra1−/− and Il20rb−/− mice compared with WT mice after PPD treatment. (A,C,E) HE staining of ear skin sections from mice, treated or not with PPD, 24 hours after the sixth application. One representative picture is shown for each treatment regimen (original magnification x30, scale bar = 50 μm). (B,D,F) Acanthosis was evaluated by measuring the epidermal thickness at six different places by using Panoramic viewer measuring tool. The percentages of crusts are calculated by dividing the length of crusts by the length of the section. These analyses were performed in Il22ra1+/+ and Il22ra1−/− 129/Sv mice (A,B), in Il20rb+/+ and Il20rb−/− C57BL/6 mice (C,D) and Il24+/+ and Il24−/− C57BL/6 mice (E,F). Data are means ± SEM (N = 5 for VC groups and N = 8 for PPD-treated groups) and representative of four independent experiments. *p < 0.05 and **p < 0.01 (Mann-Whitney to compare treated mice). Histological analysis was performed by two evaluators. VC: vehicle control.

Figure 5. IgE-dependent allergy is similar in WT and Il22ra1−/−, Il20rb−/− and Il24−/− mice after PPD treatment. Mice were treated or not with PPD and 24 hours after the third application, IgE levels in the sera were assessed by ELISA. This analysis was performed in Il22ra1+/+ vs Il22ra1−/− 129/Sv mice (left panel), in Il20rb+/+ vs Il20rb−/− C57BL/6 mice (central panel) and in Il24+/+ and Il24−/− C57BL/6 mice (right panel). Data are means ± SEM (N = 3 for VC groups and N = 6 for PPD-treated groups). VC: vehicle control.
In conclusion, in contrast to psoriasis where different IL-20 related cytokines play a role, IL-24 is the main IL-20-related cytokine playing a role in PPD-induced CHS, most probably via its effect on keratinocytes. It induces acanthosis and production of chemokines, in turn triggering a neutrophil influx that plays a crucial role in contact hypersensitivity.

Material and Methods

Patients. Eleven patients with a history of positive patch-test reaction to PPD were included in this study. The patients were otherwise healthy and only investigated when clinically in remission of their dermatitis. All of them had a history of allergic contact dermatitis after using hair dyes or after temporary black henna tattoos. The study and data accumulation were conducted with the approval of the Institutional Ethical Committee, Commission d’Ethique Biomédicale Hospitalo-Facultaire de l’Université catholique de Louvain (NCT 340320084407). All experiments were performed in accordance with relevant guidelines and regulations. Informed consent for all the diagnostic procedures was obtained from all study subjects.

All subjects were examined clinically and patch tested with para-phenylenediamine 1% diluted in petrolatum (Chemotechnique). Three series of PPD patch tests were applied. The patch-test materials used were IQ Ultra® chambers (Chemotechnique) covered on the buttocks with Fixomull stretch® (Smith and Nephew). The patch-test reactions were evaluated after 8, 24 and 48 hours according to the ICDRG criteria (Suppl. Table 1)43.

Three mm-punch biopsies from patch tests, whether positive or negative, were collected at 8, 24, and 48 hours following PPD application. Before patch testing (0 hour), normal skin was also biopsied.

PBMCs isolation and stimulation. Blood samples were collected before patch testing. Total human PBMCs were purified from the blood of control or allergic patients by centrifugation on a Lymphoprep gradient (Elitech). Cells were then washed with PBS EDTA 1 mM and resuspended in autologous medium (RPMI medium (Gibco) containing 5% of plasma patient). PBMCs were stimulated during 48 hours at 37°C with anti-CD3 anti-CD28 beads (Life, 500 000 beads for 10⁶ cells) and PPD (Sigma, 2.5 µg/mL). After this incubation, cells were harvested for RNA extraction.

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**Figure 6.** Epidermal infiltration of CD45⁺ cells decreases in Il22ra1⁻⁻ and Il20rb⁻⁻ mice compared with WT mice. Flow cytometry on epidermal cells from mice, treated or not with PPD (VC), 24 hours after the second application. (A) The percentage of CD45⁺ cells among living cells was analyzed. (B) The proportion of TCRβ⁺ cells among CD45⁺ living cells was analyzed. (C) The proportion of Ly6G⁺CD11b⁺ cells among CD45⁺ living cells was analyzed. These analyses were performed in Il22ra1⁺⁺ and Il22ra1⁻⁻ 129/Sv mice (left panels), in Il20rb⁻⁻ and Il20rb⁻⁻ C57BL/6 mouse (central panels) and Il24⁺⁺ and Il24⁻⁻ C57BL/6 mice (right panels).

Data are means ± SEM (N = 5 for VC groups and PPD-treated groups) and representative of three independent experiments. *p < 0.05 and ***p < 0.001 (Mann-Whitney to compare treated mice). VC: vehicle control.
Mice. All mice used in this study were bred in the animal facility of the Brussels branch of the Ludwig Institute for Cancer Research under specific pathogen-free conditions. Rag2−/− BALB/c mice were originally purchased from Taconic and C57BL/6 mice were purchased from Jackson Laboratory. IL22ra1−/− mice, in C57BL/6 background, were provided by U.M. Wegenka (University Medical Center, Ulm, Germany)25. Wild-type (WT) 129/Sv mice were originally purchased from Harlan. IL22−/− mice were generated in 129/Sv background in our laboratory as described previously44. IL-22R-deficient mice were generated in 129/Sv and C57BL/6 background in our laboratory as described in Suppl. Fig. 7. All mice were bred as heterozygous and littermate controls were used for in vivo experiments. The IL22−/− mice were used in 129/Sv background, IL22ra1−/− mice were used in 129/Sv or C57BL/6 background and IL24−/− and IL20rb−/− in C57BL/6 background. The experiments were performed in compliance with institutional guidelines and were approved by the Animal Research Ethical Committee of the Université catholique de Louvain (2015/UCL/MD/09). Mice between 8 and 12 weeks of age were shaved on the back skin one day before CHS triggering.

CHS model. Our CHS model is based on the timing used in Rothe et al. study26, namely PPD application at day 0, 5, 10, 11, 12 and 13. For the first and the second application, mice were treated on shaved back skin and the dorsum side of ears by applying a solution of H2O2 3% and PPD (CAS 106–50–0, Sigma), 3% [W/V] for 129/Sv back background mice and 5% for C57BL/6 and BALB/c back background mice, diluted in acetone: olive oil (4:1).
The application of PPD on ears from the first application aims to mimic what happens in PPD-allergic patients who are both sensitized and elicited at the same site. The third application is done by applying the H2O2/PPD sequence of primers and probes are listed in Suppl. Table 2. Reverse transcription and condition used for the RT-qPCR were described before. Quantitative PCR (qPCR) amplifications were performed using primer sets and TaqMan probes corresponding to murine β-actin, Il19, Il22, Il24, Ifng, Il17 and Ngp or human Il24, Ef1, Il19, Il20, Il24 with qPCR Mastemix TaqMan (Eurogentec). For murine Rlpl9, Il20, Cd3e and Krt10, qPCR was done using MasterMix for SYBR Green (Eurogentec). The sequences of primers and probes are listed in Suppl. Table 2.

**Statistics.** Results are presented as the mean ± SEM. Statistical significance between groups was assessed by using one-tailed unpaired Student t test, Mann-Whitney test in non-parametric conditions and two-way ANOVA with Bonferroni’s post-test for the ear thickening curves, using the Prism software (GraphPad software).

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**Single-cell suspension and FACS staining.** Ears were dissected and incubated overnight in dispase II at 1 U/ml (Roche) at 4°C. The epidermis and the dermis were separated as previously described. Cells were incubated with 10 µg/ml of purified rat anti–mouse CD16–CD32 monoclonal antibody (Fc Block). Then, the specific antibodies were added for 1 h at 2 µg/ml at 4°C. PerCP-labeled anti-CD45 (30-F11), APC-labeled anti-TCR/3 (H57–597), PE-labeled anti-CD11b (M1/70), FITC-labeled anti-Ly6G (1A8). A viability marker was also added (LIVE/DEAD® Fixable Near-IR Dead Cell Stain Kit, Life). Cells were gated, based on forward and side scatter, on viability marker and on living hematopoietic cells (CD45+ cells) with FACS Fortessa (BD Biosciences). Postacquisition analysis was performed using FlowJo software (Tree Star).

**Purification of CD45+ cells.** Epidermal cell suspensions from 129/Sv mice were prepared as described above. The MACS system from Miltenyi Biotec was used to isolate CD45+ cells, following the manufacturer’s instructions. Briefly, cells were incubated for 20 min at 4°C with anti-CD45 antibody-coupled microbeads, washed, and separated by two passages on the MACS instrument. Purification was checked by FACS analysis with an anti-CD45 antibody and determined to be at least 90% of purity for PPD-treated skin.

**IgE measurement.** IgE titers were measured in sera by ELISA using specific reagents from LO/IMEX, (Université catholique de Louvain). All absorbance reads were made at 450 nm, using a 96-well plate spectrophotometer.

**Histological analysis.** Paraffin tissue blocks of mouse ear skin were prepared using routine methods and consecutive sections were made. The sections were stained with HE for mouse skin and scanned with Mirax (Zeiss). Epidermal thickness was measured at different places of the section thanks to Pannoramic Viewer measuring tool (3DHISTECH). Percentages of crusts were calculated by measuring length of crusts divided by the length of the section. Two evaluators performed analysis of the staining.

**RT-PCR.** Total RNA was isolated from mouse ears or skin of patients using TriPure isolation reagent (Roche). Reverse transcription and condition used for the RT–qPCR were described before. Quantitative PCR (qPCR) amplifications were performed using primer sets and TaqMan probes corresponding to murine β-actin, Il19, Il22, Il24, Ifng, Il17 and Ngp or human Il24, Ef1, Il19, Il20, Il24 with qPCR Mastemix TaqMan (Eurogentec). For murine Rlpl9, Il20, Cd3e and Krt10, qPCR was done using MasterMix for SYBR Green (Eurogentec). The sequences of primers and probes are listed in Suppl. Table 2.

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The application of PPD on ears from the first application aims to mimic what happens in PPD-allergic patients who are both sensitized and elicited at the same site. The third application is done by applying the H2O2/PPD solution on ears only. The next applications are performed without H2O2. Control mice received vehicle solution (including H2O2 3% for the three first applications). All solutions were prepared freshly. Ear thickness was measured before each application of PPD and 24 hours after the last application with a micrometer screw (Mitutoyo). For chemokine injection, we injected 1 µg Cxcl1 and 3 µg Ccl3 (Immunotools) in ears during the first and the second applications.
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Author Contributions
A.B.V.B., P.M.C., J.-C.R., M.B. and L.D. wrote the main manuscript text and prepared the figures. A.B.V.B., P.M.C., L.P., R.O., P.C. and E.H. performed the experiments. M.D.H., P.R., Y.A. and G.W. generated the Il22ra1−/− mice. All the authors reviewed the manuscript.

Additional Information
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