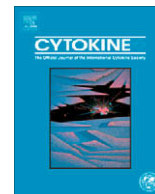




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Interleukin-22 is a negative regulator of the allergic response

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ABSTRACT

A proinflammatory role of T helper (Th)17 cells, producing IL-22 and IL-17A, has been favored although there is evidence for negative immune regulation by IL-17A. Here we show that IL-22 was produced during an allergic response in lungs of mice, immunized and challenged with ovalbumin (OVA), and that IL-22 neutralization further augmented the eosinophil recruitment to the lung. In a second allergy model, transfer of OVA-pulsed dendritic cells (DC) into naive mice conveyed eosinophil recruitment in response to subsequent inhaled OVA challenge, while DC preincubation with recombinant IL-22 abolished this response. Similarly, DC preincubation with IL-17A abolished DC-driven eosinophil recruitment, showing that both Th17 cytokines IL-22 and IL-17A mediate negative regulation of allergy by acting on DCs. Therefore, IL-22 inhibits DC functions and attenuates an allergic response.

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1. Introduction

Allergic asthma has increased dramatically in prevalence and severity over the last three decades. It is a chronic inflammatory disorder of the airways with a prevailing immune response to inhaled allergens leading to bronchial hyperreactivity, recruitment of eosinophils, mast cells and lymphocytes, hyperplasia of smooth muscle and goblet cells, often associated with increased serum immunoglobulin E concentrations [1]. T helper cells and the recently described Th17 cells play an important role in allergic lung inflammation [2]. Understanding the molecular and cellular mechanisms of action of Th17 cells in allergic asthma may lead to novel therapeutic approaches [2,3].

IL-17A and IL-17F are novel negative regulators of antigen driven Th2 response [2,4]. IL-17A has been found elevated in lungs of allergic patients [5] and antigen-specific Th17 and iNKT were described as sources of IL-17A [6]. IL-17A and IL-17F are co-expressed in Th17 cells together with IL-22 [7]. Therefore, we address the possible role of IL-22 as a negative regulator of allergic inflammation.

IL-22, originally described as an IL-10-related T cell-derived inducible factor (IL-TIF) sharing 22% identity with IL-10, belongs to the IL-10 cytokine family [8]. IL-22 binds to a membrane

receptor complex composed of the IL-10R β and IL-22R chains [9], and signals intracellularly through transcription factor STAT3 as well as MAP kinases [10].

IL-22 not only acts as a stimulator of the innate immune system by upregulating acute phase proteins and anti-microbial peptides [7] but it is also a down-modulator of the following committed immune responses. First, IL-22 provided protection to hepatocytes during acute liver inflammation [11] and protected against ConA- or Tetrachloride-induced liver injury [12]. Second, delivery of IL-22-Ig fusion gene ameliorated experimental autoimmune myocarditis (EAM) in rats [13]. Third, local IL-22 gene delivery led to rapid amelioration of intestinal inflammation in a mouse model of ulcerative colitis and, conversely, inhibition of IL-22 activity by local overexpression of its antagonist, IL-22 binding protein, prevented recovery and goblet cell restitution in acute colitis (DSS) [14]. Forth, in human cell cultures, IL-22 reduced expression of at least seven genes associated with keratinocyte differentiation [15,16].

IL-22 is expressed by IL-9-activated mast cells and Th17 cells initiated by TGF- β in the context of IL-6 and other proinflammatory cytokines [7]. IL-22, similar to IL-17A [17–20], has been found in diseased tissues from patients with different chronic inflammatory diseases, involving infiltrating activated T cells, such as rheumatoid arthritis, psoriasis, inflammatory bowel disease and COPD [21–25].

The professional antigen presenting cells, the DC, represent a key target of immunoregulatory cytokines. IL-10, the prototype of the IL-10 family, inhibits DC functions resulting in a decreased Th2 response upon antigen-pulsed DC transfer to naive mice [26]. IL-17A reduces DC cells chemokine production [2]. Therefore, IL-17A, much like published data on IL-10 [27–29], is recognized as negative regulator of the allergic response.

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To address whether IL-22 also affects DC functions in the allergic model, we investigated the role of IL-22 on eosinophil recruitment in experimental models of pulmonary allergic inflammation induced by either subcutaneous OVA-immunization or OVA-pulsed DC transfer to naive mice followed by OVA inhalation challenge.

2. Material and methods

2.1. Material

O-Phenylenediamine, 3-amino-1,2,4-triazole, horseradish peroxidase, BSA grade V, Ovalbumin and aluminum hydroxide were obtained from Sigma Chemical Company (St. Louis, MO). The antibodies used for FACS analysis, PE-anti-CD40 (clone 3/23), APC-anti-CD80 (clone 16-10A1), PerCP-anti-CD86 (clone GL1), and isotype-matched controls were purchased from Pharmingen (San Diego, CA). α Galactosyl Ceramide (α GC) was from Kirin Inc. (Japan). Anti-IL-22 antibody was obtained from Abcam (USA). Anti-IL-17A neutralizing monoclonal antibody were provided by Schering Plough (Dardilly, France) and R&D (Abingdon, UK). Recombinant mouse IL-23, IL-17A, IL-17F, IL-22 and TSLP were purchased from R&D (Abingdon, UK).

2.2. Mice

Balb/c wild type mice, receptor IL-4R α KO were bred in our specific pathogen free animal facility at CNRS, Orleans, France. Receptor IL-4R α KO mice were on Balb/c genetic background as described previously [30]. Mice were maintained in a temperature-controlled (23 °C) facility with a strict 12 h light/dark cycle and were given free access to food and water. The experiments were performed with gender-matched mice aged 8–10 wk. All protocols complied with the French Government's ethical and animal experiment regulations.

2.3. Experimental protocol

Mice were immunized subcutaneously twice at weekly intervals with 0.4 ml saline containing 10 μ g Ovalbumin (OVA¹) with the 1.6 mg adjuvant aluminum hydroxide as previously described [2]. One wk after the second immunization, mice were challenged two times (at days 14, 16) with OVA (1% in water) by inhalation during 20 min. Control mice were challenged with saline alone. Two days after the last challenge mice were sacrificed and the lungs analyzed.

Mice were given a high dose of Ketamine/Xylazine i.p. and bled out. Lungs were washed two times with 1 ml of ice-cold saline solution using a canula which was inserted via tracheal puncture. After bronchoalveolar lavage, the lung was perfused via heart puncture with ISOTON[®] II Acid free balanced electrolyte solution (Beckman Coulter, Krefeld, Germany). Half of the lung was stored at –80 °C for EPO enzyme and chemokine analysis and the other half was fixed overnight in buffered 4% formaldehyde solution for histology analysis. Mediastinal lymph nodes (LN) were removed for cell cultures and FACS analysis. BAL fluid was analyzed for cell composition and cytokine concentrations. Experiments were performed at least twice each using groups of 4 animals.

2.4. Bronchoalveolar lavage (BAL)

Bronchoalveolar lavages (BAL) fluids were prepared by washing the lungs twice with 1 ml of saline solution at room temperature.

The cells were sedimented by centrifugation at 400g for 10 min at 4 °C. The supernatant (cell-free BAL fluid) was stored at –20 °C for cytokine analysis. An aliquot of the cell pellets was stained with Trypan blue solution, counted, and 200,000 cells centrifuged on microscopic slides (cytospin at 1000 rpm for 10 min, at RT). Air-dried preparations were fixed and stained with Diff-Quik (Merz & Dade A.G., Dudingen, Switzerland). Differential counts were made under oil immersion microscopy at $\times 80$ magnification. One hundred cells were counted twice for the determination of the relative percentage of each cell type present in the BAL.

2.5. Pulmonary eosinophil peroxidase (EPO) activity

EPO activity was determined in order to estimate the recruitment of eosinophils to the lung parenchyma. After BAL lavage and perfusion (see experimental protocol), the lungs were excised, stored frozen at –80 °C or directly homogenized for 30 s in 1 ml of 0.05 M Tris/HCl-buffer pH 8.0 using a Polytron (Kinematic AG, Luzern, Switzerland). The homogenate was centrifuged for 15 min at 4 °C at 10,000g. EPO activity in the supernatant was determined as estimated from the oxidation of O-phenylenediamine (OPD) by EPO in the presence of hydrogen peroxide (H₂O₂) using the protocol by Van Oosterhout and colleagues [31]. The substrate solution consisted of 10 mM OPD in 0.05 M Tris/HCl-buffer (pH 8) and 4 mM H₂O₂ (BDH, Poole, UK). Substrate solution was added to samples in a 96-wells micro plate (Greiner) and incubated at 37 °C for 30 min. Duplicate incubations were carried out in the absence and presence of the EPO inhibitor 3-amino-1,2,4-triazole (AMT, 2 mmol/l). The absorbance was then measured at 490 nm (Flow Labs, Irvine, UK). Results are expressed as OD 490 nm and were corrected for the activity of other peroxidases, which were not inhibited by AMT.

2.6. Quantification of IL-17A, IL-22, IFN, IL-5, IL-10, CCL5, CCL11, and CCL17

Cytokine and chemokine concentrations in BAL, lung homogenate, or cell culture supernatants, were determined by enzyme-linked immunosorbent assay (ELISA), using commercial kits from R&D (Abingdon, UK). The lungs were homogenized for 30 s using a Polytron (Kinematic AG, Luzern, Switzerland) and the cell debris were eliminated by centrifugation at 10,000g for 15 min. The chemokines were determined in the supernatants. The cytokine detection limit was 1 pg/ml.

2.7. Lung explants and lymph node cell cultures

Lungs were excised, minced and passed through 100 μ m pore size filters, and lymph nodes (mediastinal) were taken into suspension by passing them through 100 μ m mesh. The cells were washed once with saline by centrifugation for 10 min at 1300 rpm, counted, and the cell pellet was resuspended at 10⁶ cells per ml in culture medium consisting of DMEM supplemented with 10% FCS and 100 U/ml Penicillin/Streptomycin for lung cells, and consisting of RPMI supplemented with 10% FCS, 100 U/ml Penicillin/Streptomycin and 2-ME for LN cells. The cells were cultivated in 24 well plates as a 1 ml cell suspension per well, with the indicated stimuli for 3 days at 37 °C in a humidified incubator with 5% CO₂. Stimulations were with 50 μ g/ml OVA, 100 ng/ml α Galactosyl Ceramide (α GC), and 100 ng/ml to 10 μ g/ml recombinant CCL5.

2.8. Bone marrow derived DC transfer to naive mice

Murine bone marrow cells were isolated from femurs and differentiated into myeloid DCs by culturing at 2 $\times$ 10⁵ cells/ml for 10 days in RPMI medium supplemented with 10% FCS, non-essential amino-acids, 0.05 μ g/ml Asparagine, MEM Vitamins, sodium pyru-

¹ Abbreviations used: BAL, bronchoalveolar lavage; DC, dendritic cells; EPO, eosinophil peroxidase; mLN, mediastinal lymph node; OVA, ovalbumin.

vate, Gentamycin 50 µg/ml, 10,000 U/ml Pen, 10 mg/ml Strep, 2-mercaptoethanol 50 µM and 4% J558L cell-conditioned medium as a source of GM-CSF (change medium on days 3, 6, and 8) as described previously [2]. For DC transfer experiments, 10^6 cells were treated for 18 h with 50 µg/ml OVA in medium containing 10% FCS in the absence or presence of IL-22 or IL-17A, respectively. The phenotype of the DCs is given in Fig. 2. Non-adherent cells were harvested, washed twice in saline solution, and 10^6 DC were injected intravenously into naïve mice. In control experiments, cells were left untreated and injected intravenously. At days 8, 9, 10 after DC transfer mice were nebulized with 1% OVA solution for 20 min. At day 11 after DC transfer mice were sacrificed and analyzed.

2.9. Dendritic cell uptake of OVA-FITC

One hundred µg/ml of OVA-FITC was added to dendritic cell cultures at day 12. Cells were incubated for 2 h at 37 °C in a humidified incubator with 5% CO₂. Cells were collected, washed three times with cold PBS containing 1% BSA and analyzed using a FACS-Calibur-Flow cytometer and CellQuest Software (Becton-Dickinson, San Jose, CA). The markers for the monovariant histogram (not shown) were set based on the negative staining control, which was provided by incubating dendritic cells with OVA-FITC at 4 °C.

2.10. Statistical analysis

The data are presented as the means ± SD with $n = 8$ animals per condition. The significance of differences between two groups was determined by Student *T*-test (two-tailed). Statistical significance was reported if $P < 0.05$ was achieved.

3. Results

3.1. IL-22 expression in allergy and its suppression by IL-4 signaling

We showed recently a correlation of pulmonary IL-23 and IL-17A production in allergic lungs [2]. Here we demonstrate that IL-22 is also present in lung homogenates of OVA sensitized and challenged mice (Fig. 1B). Ex vivo stimulation of mediastinal lymph node (mLN) cells isolated from antigen OVA sensitized and challenged mice with IL-23 triggered IL-22 production which was even further enhanced in the presence of OVA (Fig. 1A).

Since IL-4 affects the development of Th17 cells in vitro and in vivo [32,33] and we demonstrated that IL-17A production is increased in the absence of IL-4Rα signaling [2], IL-22 production was analyzed in IL-4Rα KO mice. IL-22 production upon OVA challenge was significantly increased by 35% ($P = 0.011$) in IL-4Rα KO mice compared to WT mice (Fig. 1B). Pulmonary OVA-induced IL-23 concentrations were equal in IL-4Rα KO and WT mice as we have demonstrated recently [2]. Therefore, the IL-4 signals affect directly IL-22 production and do not act on IL-23 production [2].

3.2. IL-22 down-regulates eosinophil recruitment

We then investigated the functional role of IL-22 using the high IL-22 expressing IL-4Rα KO mice (Fig. 1B). Blocking endogenous IL-22 by neutralizing antibodies during the antigen challenge recovered both the eosinophil recruitment to the airways and eosinophil peroxidase (EPO) activity in the lungs of IL-4Rα KO mice (Fig. 1C and D). This suggests that the restrained allergic response in IL-4Rα KO mice is partially due to endogenous IL-22 inhibiting the eosinophil response.

In WT mice neutralization of endogenous IL-22 also exacerbated the allergic response (Fig. 1C and D). Exacerbation was less

pronounced which correlates with the lower expression of endogenous IL-22 (Fig. 1B) in WT compared to IL-4Rα KO mice.

In contrast, the enhanced neutrophil recruitment seen in challenged IL-4Rα KO mice was not reversed, but rather further increased after neutralization of endogenous IL-22 (Fig. 1E). This indicates that IL-22 may also be a down-modulator of neutrophil recruitment, unlike IL-17A, which recruits neutrophils [2]. Therefore, these data suggest that endogenous IL-22 produced in the effector phase of allergic response has a novel down regulatory role.

3.3. IL-22 inhibits DC activation

We investigated the effect of recombinant IL-22 on DCs. Bone marrow derived DCs were incubated with OVA in the absence or presence of recombinant IL-22. The OVA-uptake by DC was reduced by IL-22, although the effect was small, about 10–15% (Fig. 2A). IL-22 reduced co-stimulatory molecule expression on DC upon OVA stimulation. Upregulation of CD80 was reduced by 82%, of CD86 by 75%, and of CD40 by 26% on DC by IL-22 incubation (Fig. 2B–D). For comparison, IL-17A reduced CD86 and CD40 induction by 19% and 35%, respectively (Fig. 2E and F). These data implicate that IL-22 and IL-17A reduce the OVA-induced activation of DCs providing a mechanism to reduce the initiation of the Th2 response.

3.4. IL-22 reduces the in vivo response of OVA-pulsed DC

In order to test whether reduced DC co-stimulatory molecule expression resulted in a diminished antigen sensitization, naïve mice were sensitized through injection of OVA-pulsed DCs, which had been pulsed in the presence or absence of IL-22. Mice were then challenged by inhaled OVA. Pulmonary eosinophil recruitment was increased 8-fold in mice treated with OVA-pulsed DCs compared to mice treated with unpulsed DCs (Fig. 3A and D). Treatment of the DCs with IL-22 during the OVA-pulsing reduced eosinophil recruitment to the bronchoalveolar space by 79% (Fig. 3A). The reduced recruitment correlated with diminished eosinophil peroxidase activity in the tissue (Fig. 3B) and Th2-type chemokine CCL17/TARC production (Fig. 3C). For comparison, treatment of DCs with recombinant IL-17F resulted in mild reduction of eosinophil recruitment to the airways (BAL; Fig. 3A) while recruitment to the tissue was strongly reduced by 71% (Fig. 3B). Conversely, incubation of DCs with recombinant IL-17A reduced eosinophil recruitment to the airways (BAL) and tissue by 95% and 64%, respectively (Fig. 3D and E). As a positive control, DCs were co-incubated with OVA and TSLP (Thymic stromal lymphopoietin), a recognized DC activator in an allergic response [34]. OVA/TSLP treated DCs increased the eosinophil recruitment 2-fold (Fig. 3D) compared to OVA treated DCs. The reduced eosinophil recruitment by IL-17F and IL-17A treated DCs correlated with reduced pulmonary chemokine CCL17 production (Fig. 3C and F). These data implicate that the Th17 derived cytokines IL-22, IL-17F and IL-17A can affect DC functions and reduce the OVA driven Th2 response in vivo.

3.5. IL-22-reduced Th2 response is not due to increased Th1 response

To ask whether IL-22 specifically skewed Th2 response, Th polarization was analyzed. Mediastinal lymph node (mLN) cells from mice treated with OVA-pulsed DCs, pulsed in the presence or absence of IL-22 were collected two days after the last OVA challenge and restimulated ex vivo with OVA (Fig. 4). LN cells from mice treated with OVA-pulsed DCs produced the cytokines IL-5 (Th2), IL-17A (Th17) or IFNγ (Th1) upon restimulation. However, LN cells from mice treated with DCs that had been OVA-pulsed

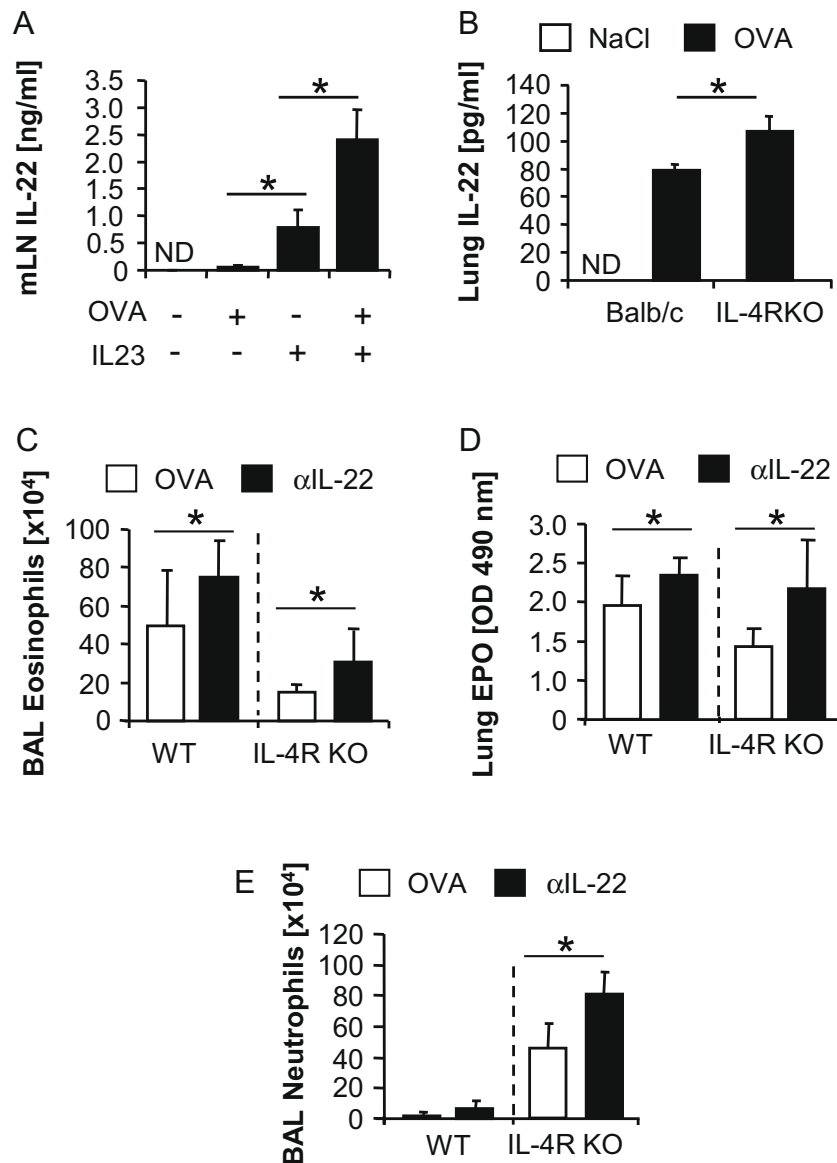


Fig. 1. IL-22 is induced in the lung upon allergen challenge. Mediastinal lymph nodes (MLN) of OVA-sensitized and challenged mice (as indicated) were restimulated in vitro with 10 ng/ml IL-23, 50 μ g/ml OVA, or both together, or medium alone, and IL-22 production was determined by ELISA. In (B), IL-22 was determined in lung homogenate of OVA/Alum-sensitized and OVA-challenged WT mice (Balb/c) or IL-4R α KO mice. In (C–E), mice were challenged with OVA/IgG or with OVA/anti-IL22 antibodies (10 μ g/mouse) and 48 h after the last OVA challenge eosinophil (C) and neutrophil (E) counts were determined in the bronchoalveolar space. Eosinophil peroxidase activity (EPO) in the lung tissue homogenates (D) was given in optical density (OD 490 nm) values. The bars represent the mean $\pm$ SD of two independent experiments (total of $n = 8$ animals per group). “nd” stands for not detected.

in the presence of IL-22 produced 89% less IL-5, 26% less IL-17A and 25% less IFN γ . Therefore, IL-22-induced reduction of the Th2 response is due to a restrained immune response including reduced Th1, Th2 and Th17. Since the reduction of Th1 response is less pronounced than Th2 reduction this may manifest in a respectively augmented Th1 response further accounting for the reduced allergic response. For comparison, LN cells from mice treated with DCs OVA-pulsed in the presence of IL-17A also produced 67% less IL-5, 72% less IL-17A and 40% less IFN γ . These data indicate that the Th17 cytokines reduce Th2 response through dampened DC activation and immune response.

4. Discussion

Here we demonstrate that IL-22 acts as a negative regulator in lung inflammation in mice. IL-22 reduces the hallmarks of the

allergic response, and eosinophil recruitment. IL-22 is present in lungs during allergic inflammation and neutralization of IL-22 exacerbates the allergic response. Addition of recombinant IL-22 prevented DC activation and antigen (OVA) sensitization induced by transfer of ex vivo OVA-loaded DC. This role has previously been ascribed to the prototype immune regulator IL-10 [26]. Here we show that the Th17 cytokines, IL-22 as well as IL-17 (IL-17A) and IL-17F, dampened antigen-activation of DCs resulting in a reduced Th2 response in the effector phase in vivo.

On the molecular and cellular level, it has previously been published that IL-22 has beneficial effects by enhancing hepatocyte survival, expression of transcription factor STAT3, Bcl-2, and Bcl-xL in inflamed liver and colon [11,12]. Further, evidence has been provided that IL-22 has anti-inflammatory effects through inhibition of COX-2, IL-6, chemokine production in fibroblasts or endothelial cells in autoimmunity [13], and inhibition of cell cycle in the G2-M phase and intracellular AKT kinase phosphorylation in

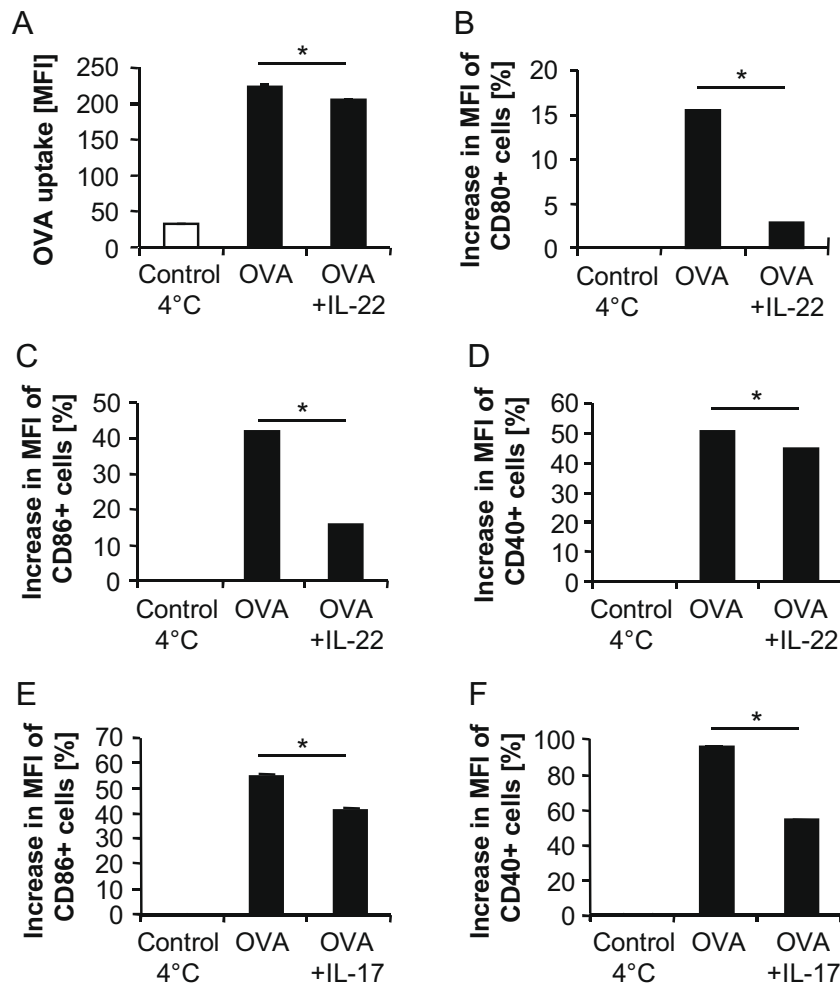


Fig. 2. IL-22 reduced OVA-uptake by DCs and OVA-induced co-stimulatory molecules. DCs were differentiated in vitro from naive bone marrow derived cells. In (A) the uptake of 100 μ g/ml OVA-FITC by DCs after 12 h in the absence or presence of 20 ng/ml IL-22 was analyzed by FACS. The data are given as the mean fluorescence intensity (MFI). Cells were treated overnight with saline, OVA, or OVA together with 20 ng/ml IL-22, or OVA together with 30 ng/ml IL-17 and co-stimulatory molecules CD80, CD86 and CD40 were determined by FACS. The data are given in % increase in MFI compared to saline treated cells. The absolute MFI values of untreated cells (control) were 25 for CD80 (B), 38 for CD86 (C + E), and 166 for CD40 (D + F). Addition of IL-22 or IL-17A in the absence of OVA had no effect on the expression of the co-stimulatory molecules. The results represent the mean $\pm$ SD of $n = 4$ animals per group.

human tumor cells in vitro [16]. Here we show inhibition of DCs and their expression of co-stimulatory molecules upon antigen treatment resulting in reduction of hallmarks of an allergic Th2 response in vivo. Therefore, our findings from experimental models of allergic lung inflammation together with the data of others with experimental models of autoimmunity, and inflamed liver and colon corroborate a novel negative regulatory function of IL-22.

An understanding of the biological functions of IL-22 is slowly emerging. Previous studies revealed an anti-infectious role for IL-22. Thereby IL-22 induced anti-microbial peptides such as human β -defensin 2, β -defensin 3, calcium-binding proteins S100A7, S100A8 and S100A9 in skin keratinocytes [7,21]. The question arose whether IL-22 is further involved in neutrophil recruitment and may thus further contribute to the infectious defense. Neutrophil involvement has been ascribed to both infectious disease and allergic inflammation [35–37]. In allergy, neutrophil recruitment is in part due to endogenous IL-17A as we have shown previously [2,38], but not due to IL-22, since neutralization of IL-22 did not diminish neutrophil recruitment in the current allergic response.

Here, we provide evidence for the various Th17-derived cytokines including IL-22, IL-17A, and IL-17F to negatively regulate the allergic response by dampening DC-driven eosinophil recruitment. Our data are supported by recent findings which propose a negative

regulatory role of Th17 cells in experimental autoimmune encephalomyelitis (EAE) [39]. Full acquisition of pathogenic function in EAE by effector Th17 cells is mediated by IL-23. However, stimulation of the myelin-reactive T cells with TGF- β plus IL-6, instead of IL-23, completely abrogated pathogenic function of Th17 cells despite upregulation of IL-17A production [39], mainly due to their production of IL-10. However, the molecular mechanism of IL-22 expression, being either IL-23 dependent or independent, in allergic lung inflammation needs to be investigated in future experiments. Another protective role for IL-22 was found in a mouse model of Th2-mediated colitis. In this model local IL-22 gene delivery resulted in a rapid attenuation of the colitis [14]. Similar in dextran sodium sulfate (DSS)-induced colitis model, anti-IL-22 treatment led to a significant delay of recovery [14]. Therefore, even though IL-22 is an upregulator of proinflammatory gene expression and has proinflammatory function, there is growing evidence that IL-22 has, similar to IL-17A, a protective role in inflammatory diseases such as colitis, EAE and allergic lung inflammation.

IL-17A and IL-22 are both co-expressed in Th17 cells [7], yet they are induced through independent pathways. Recent studies showed that IL-22 but not IL-17A and IL-17F production, strongly depend on aryl hydrocarbon receptor signals [40]. Furthermore, IL-22 production is induced in absence of IL-6, while IL-17A pro-

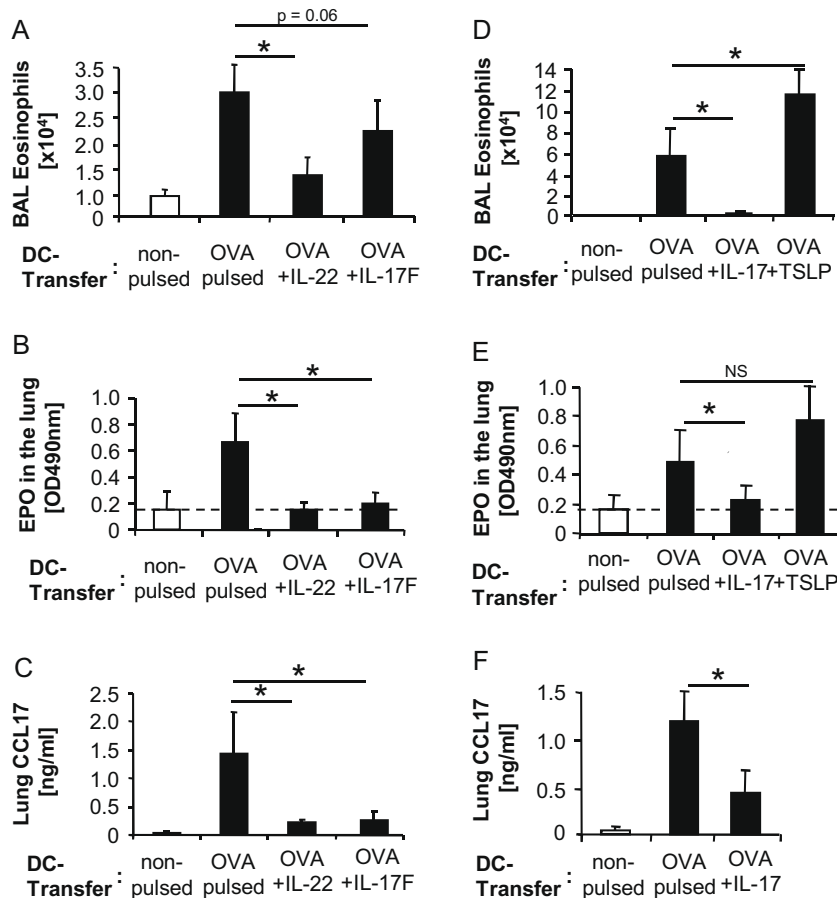


Fig. 3. Th17 cytokines-treated DCs are unable to mount a full allergic lung inflammation. Naive WT mice were sensitized to OVA through injection of OVA-pulsed bone marrow derived DC which pulsed either in the presence of saline, 20 ng/ml IL-22, 100 ng/ml IL-17F, 30 ng/ml IL-17A, or 100 ng/ml TSLP. Eight days later mice were challenged three times with OVA by inhalation. Forty-eight hours after the last challenge eosinophil recruitment to the bronchoalveolar space (A and D) was determined by counting and lung eosinophils were estimated by EPO measurement (B and E). The eosinophil peroxidase activity (EPO) in the lung tissue homogenates was given in optical density (OD 490 nm) values. CCL17 production in the lung tissue (C and F) was determined by ELISA. DCs treated with IL-22 or IL-17 in the absence of OVA showed the same results as untreated DCs. The results represent the mean $\pm$ SD of two independent experiments (total of $n = 10$ animals per group).

duction depends on the presence of IL-6 [22]. Additional IL-22 production is ascribed to IL-9 activated mast cells [8]. We found that IL-17A, but not IL-22 production, was markedly dependent on the intracellular signaling of the IL-1 receptor and its adaptor MyD88 pathway in lymph node cell cultures originating from OVA-treated mice (unpublished data). Therefore, in the allergic situation, both IL-17A and IL-22 are induced by the antigen OVA yet through distinct IL-1 dependent and IL-1 independent pathways. Together this suggests collaborative and non-redundant pathways leading to Th17 cytokines and allergy.

DCs stimulated with OVA in the presence of either cytokine, IL-17A, IL-22, but also IL-17F, dampened the allergic response and resulted in a reduced inflammation. These findings may provide a possible explanation why Yang and colleagues found an increased allergic response in IL-17F deficient mice [4].

We have previously published that IL-17A inhibited allergen induced DC functions and allergic lung inflammation [2]. IL-17A inhibited TARC production of dendritic cells, a Th2 chemoattractant, and inhibited dendritic cell activation and antigen uptake, leading to reduced IL-4, IL-13, and IL-5 production and reduced allergic response [2]. Previously we also confirmed its reported role in initiating allergic asthma, using IL-17R gene deficient mice [2]. This dual role of IL-17A is likely not mediated through the effect of IL-17A on DC functions but rather on IL-17A-induced neutrophil recruitment in the allergic response. These proallergic and inflammatory roles could be explained by IL-17A inducing hematopoietic

Colony-Stimulating Factors [41], and production of neutrophil-chemokines KC and IL-8 (CXC) [42–45]. In the current study we consolidated the negative regulatory effect of IL-17A on dendritic cells and the allergic response showing that IL-17A dampened OVA-induced co-stimulatory molecule expression on DCs and in vivo functions of DCs in the allergic response. Furthermore, novel similar functions are attributed to IL-22. IL-22 also dampened OVA-induced co-stimulatory molecule expression on DCs and in vivo functions of DCs leading to a reduced allergic response.

Neutralization of Th2 cytokines is a therapeutic strategy to reduce allergic asthma by using neutralizing IL-4 and IL-13 antibodies or soluble IL-4R α molecules [30,46,47], as confirmed by a reduced Th2 response in mice lacking receptor IL-4R α or transcription factor Stat6 [48,49]. In parallel the IL-4R α KO mice showed increased IL-22 production, whose neutralization by antibodies caused elevated eosinophil infiltration in the allergic response. Therefore, intervention at the IL-4 signaling expectedly reduced eosinophil response but beyond that upregulated IL-22 which further reduced eosinophil recruitment by targeting DC functions. Therefore, we provide an additional mechanistic understanding of the proallergic effects mediated by IL-4R α signals, through IL-22.

In conclusion, Th17-derived IL-22 negatively regulated allergy and DC functions. Its expression was further induced by allergic stimulation together with IL-23 and was controlled by IL-4R α signals. While neutralizing IL-22 antibodies augmented the allergic response, IL-22 reduced the response by inhibiting DC functions.

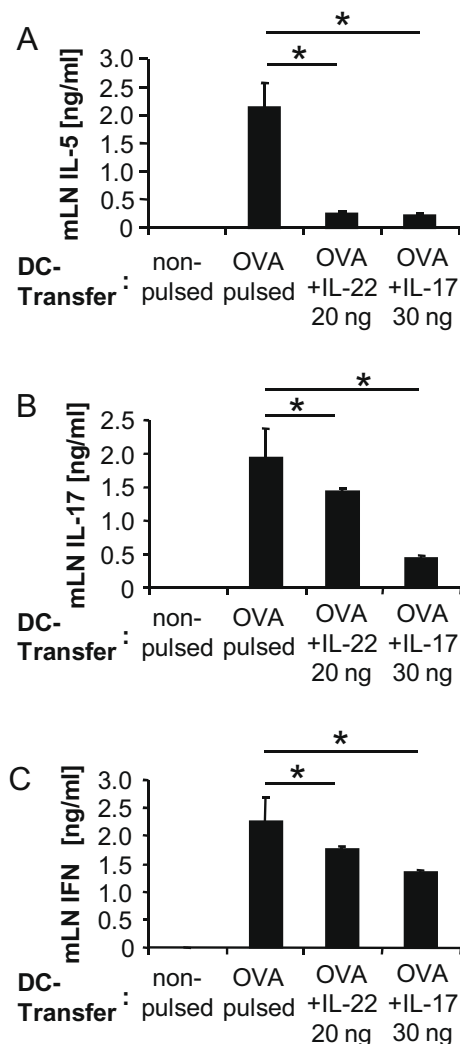


Fig. 4. Mediastinal LN excised from mice sensitized with IL-22- or IL-17A-treated DCs mounted a reduced immune response. Mediastinal lymph nodes were excised, from mice that had been sensitized with OVA-pulsed DCs in the presence of IL-22, or IL-17A, or in their absence, 48 h after the last challenge. Cells were restimulated either with saline or with 50 µg/ml OVA, IL-5 (A), IL-17A (B) and IFN (C) production was determined by ELISA 72 h later. The results are representative for two independent experiments, showing the mean ± SD of $n = 4$ animals per group.

Therefore, the data demonstrate that IL-22 is a novel endogenous negative regulator of allergy and IL-22 or Th17 cells may represent an interesting therapeutic target in lung allergy.

Disclosures

The authors have no conflicting financial interests.

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