

# Endogenous interleukin-10 suppresses allergen-induced airway inflammation and nonspecific airway responsiveness

K. G. TOURNOY, J. C. KIPS and R. A. PAUWELS

Department of Respiratory Diseases, Gent University Hospital, Gent, Belgium

## Summary

**Background** The airway inflammation observed in asthma is orchestrated by activated Th-2 lymphocytes relevant for the induction of altered airway responsiveness. An increasing body of evidence is accumulating that not only the pro-inflammatory cytokines interleukin (IL)-4 and IL-5 but also the immunomodulating cytokines IL-12 and possibly IL-10 are crucial for regulating the allergic airway inflammation.

**Objective** Since IL-10 is capable of downregulating a broad spectrum of pro-inflammatory cytokines, we wanted to address the role of endogenously produced IL-10 *in vivo* in allergic asthma.

**Methods** Knockout (IL-10<sup>-/-</sup>) mice (C57BL/6-IL10tm1Cgn) and wild-type (WT) counterparts were immunized (day 0) and exposed (day 14–21) to ovalbumin (OVA). Airway inflammation and reactivity (AR), serum allergen-specific IgE responses and cytokine profiles in the bronchoalveolar lavage fluid (BALF) were studied.

**Results** The IL-10<sup>-/-</sup> mice had more eosinophilic airway inflammation but comparable levels of allergen-specific serum IgE compared to the WT mice after allergen challenge. The AR was comparably increased in the OVA challenged WT and IL-10<sup>-/-</sup> mice vs sham-exposed WT, but not vs sham-exposed IL-10<sup>-/-</sup> mice since these showed a higher baseline AR. IFN  $\gamma$ , IL-4 and IL-13 were comparable and IL-5 was even lower in the BALF of the in IL-10<sup>-/-</sup> mice compared to the similarly exposed WT mice.

**Conclusion** These results indicate that IL-10 plays an important and possibly direct role in the control of airway inflammation and responsiveness in an *in vivo* mouse model of allergy.

**Keywords:** airway inflammation, airway responsiveness, eosinophil, IL-10

*Clinical and Experimental Allergy*, Vol. 30, pp. 775–783. Submitted 1 March 1999; revised 15 May 1999; accepted 22 June 1999.

## Introduction

The pathological basis of the reversible airway obstruction and airway hyperresponsiveness in bronchial asthma is inflammation. Even patients with mild asthma have inflamed airways [1] and some studies suggest that the severity parallels the degree of inflammation [2,3]. Asthmatic airway inflammation is characterized by infiltration of the mucosa by CD4<sup>+</sup> T cells of the Th-2 phenotype [4] and by eosinophils [5]. These Th-2 cells produce IL-4 and IL-5, which are thought to be relevant for the persistence of the inflammation and for the mechanism linking the airway

inflammation to the altered airway responsiveness [6]. Next to IL-4 and IL-5, other cytokines such as IL-10 are produced by Th-2 cells [7,8]. The initial description of IL-10 as a Th-2 cytokine inhibiting Th-1 proliferation was followed by studies indicating that IL-10 also inhibited cytokine production of Th-1 and Th-2 cells [9]. IL-10 has been described as an extremely pleiotropic cytokine not only inhibiting cytokine synthesis but also having stimulatory effects especially on B cells with induction of  $\gamma$ -isotype switching and IgG production [9]. Different studies performed in asthmatic patients suggested a role for IL-10 in the pathogenesis of asthma although its exact role is not yet understood [10–12].

In this study, we addressed the role of IL-10 in allergic airway inflammation using IL-10-deficient mice (IL-10<sup>-/-</sup>). Our results indicate that the absence of endogenous IL-10

Correspondence: K. Tournoy, Gent University Hospital, Department of Respiratory Diseases, De Pintelaan 185 (7 K12 IE), 9000 Gent, Belgium.

strongly enhances antigen-induced eosinophil influx and airways inflammation and increases airway responsiveness. Furthermore, we investigated the mechanisms by which IL-10 induces these findings by means of histological analysis on the pulmonary tissues and by analysis of cytokine profiles in the bronchoalveolar lavage fluids.

## Methods

### Animals

Specific pathogen-free male C57BL/6J wild-type (WT) mice and homozygous IL-10-deficient mice (IL-10<sup>-/-</sup>) (C57BL/6J-IL-10(tm1Cgn)), 6–8 weeks of age, were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA). Mice homozygous for the IL-10 gene mutation do not produce IL-10 and completely lack IL-10 activity [13]. Mice were housed in sterilized germ-free micro-isolator cages and fed with sterile food and water *ad libitum*. Bacterial cultures, parasitological examinations, serological tests were negative for known murine pathogens.

### Immunization and exposure of mice

On the first day of the experiments (day 0), all mice ( $n = 9$ –16 per group) were actively immunized with ovalbumin (OVA) (Sigma, St Louis, MO, USA) by i.p. injection of 10 µg OVA, adsorbed to 1 mg of aluminium hydroxide. From day 14 to day 21, the mice were exposed daily to phosphate-buffered saline (PBS) or OVA aerosols (1%) for 30 min in a sterilized exposure chamber (30 × 20 × 15 cm). The aerosol was produced by an ultrasonic nebulizer (Ultraschallvernebler Sirius Nova, Heyer Medizintechnologie, Bad Ems, Germany). According to the specifications of the manufacturer, the output of the nebulizer was 3 mL/min and the mean particle size of the aerosol was 3.2 µm.

### Airway responsiveness

Airway responsiveness to carbachol was measured 24 h after the final allergen exposure as previously described [14]. The mice were anaesthetized with pentobarbital (100 mg/kg i.p.) and a tracheal cannula was inserted. The femoral artery and the jugular vein were cannulated. The animals, placed on a 37 °C heated blanket, were ventilated with a Palmer respirator (Bioscience, Sheerness, UK) at 145 strokes/min (stroke volume of 0.5 mL). Neuromuscular blockade to prevent spontaneous respiration was induced by injecting pancuronium bromide (1 mg/kg) i.v. (Organon, Teknika NV, Turnhout, Belgium). Airway resistance ( $R_L$ ) was calculated from the differential pressure between the airways and the pleural cavity, tidal volume and flow. Measurements were performed with a computerized pulmonary mechanics analyser

(Mumed PMS800 system; Mumed Ltd, London, UK). Increasing doses of carbachol were administered (micro-infusion pump) intravenously (40, 120, 400 and 1200 µg/kg). Between each dose, the airway resistance was allowed to return to the baseline level. The provocative dose of carbachol causing a 50% increase in  $R_L$  ( $PD_{50-R_L}$ ) was calculated from the linear interpolation on a semilogarithmic dose–response curve.

### Bronchoalveolar lavage

Immediately after the assessment of airway responsiveness, 1 mL of Hank's balanced salt solution (HBSS) free of ionized calcium and magnesium but supplemented with 0.05 mM sodium EDTA was instilled four times via the tracheal cannula and recovered by gentle manual aspiration. The recovered bronchoalveolar lavage fluid (BALF) was centrifuged (600 g for 10 min at 4 °C). The supernatant was processed for cytokine assessment. The cell pellet was washed twice and was resuspended in 1 mL of HBSS. A total cell count was performed in a Bürker chamber and the differential cell counts on at least 400 cells were performed on cytocentrifuged preparations (Cytospin 2; Cytospin, Shandon td., Runcorn, Cheshire, UK) using standard morphological criteria after staining with May-Grünwald-Giemsa.

### Cytokine measurements in BALF supernatant

The supernatant of the BALF was passed through a 0.2-µm filter to remove mucus. A protease inhibitor cocktail, composed of soybean trypsin inhibitor,  $\alpha$ 1-antitrypsin, pepstatin A, EDTA, benzamidine hydrochloride, aprotinin and phenanthroline (Sigma Chemical Company, Poole, UK) was added (1 µL/mL supernatant). The BALF supernatant was concentrated 5–8 times by means of Centriprep 3000 concentrators (Amicon, Beverly, USA) according to the manufacturer's instructions. Centrifugation was performed at 4 °C (2600 g) for three times 30 min, discarding the low molecular weight fraction after each centrifugation. By dividing the original supernatant volume by the final volume of the concentrate, a concentration factor was obtained. The concentrated supernatant was stored at –80 °C until analysis. The cytokines IL-4, IL-5, IL-13 and IFN  $\gamma$  were measured with commercially available sandwich ELISAs (Biosource Europe, Fleurus, Belgium for IL-4, and IFN  $\gamma$ , R & D Systems Europe, Abingdon, UK for IL-13 and Biotrak, Amersham Pharmacia Biotech Benelux, Brecht-St. Lenaarts, Belgium for IL-5). Sensitivity of the ELISA kits was 1, 1.5, 5 and 5 pg/mL for IFN  $\gamma$ , IL-13, IL-4 and IL-5, respectively. The measured cytokine concentrations were divided by the concentration factor in order to obtain the original concentration of the cytokine in the BALF.

### Measurement of total and allergen-specific serum IgE

At the end of the experiment, blood was drawn from the heart for measurement of total and OVA specific serum IgE. Total IgE was measured with a two-sided sandwich ELISA assay, using two monoclonal rat antimouse IgE antibodies reacting with different epitopes on the epsilon heavy chain (H. Bazin, Experimental Immunology Unit, UCL, Brussels, Belgium). The second antibody was biotinylated and detected colourimetrically after adding horseradish peroxidase-streptavidine conjugate. Absorbance values, read at 450 nm were converted to concentrations in serum by comparison with a standard curve obtained with mouse IgE of known concentration (H. Bazin).

For OVA-specific serum IgE, microtitre plates were coated with OVA. Serum was added followed by a biotinylated polyclonal rabbit antimouse IgE (S. Florquin, ULB, Brussels, Belgium). A serum pool from OVA-sensitized animals was used as the internal laboratory standard; 1 unit was arbitrarily defined as a 1/100 dilution of this pool.

### Histological analysis and scoring of pulmonary inflammation

The lungs were infused via the trachea with 4% paraformaldehyde. After excision, the lungs were immersed in fresh fixative overnight. Pieces from all lung lobes were used for histological examination. Sections of 2.5 µm thickness were cut and stained with haematoxylin and eosin or with haematoxylin and congo red (for the photomicrographs). Slides were coded and were graded in a blinded fashion using a reproducible scoring system described elsewhere [15]. A value from 0 to 3 per criterium was adjudged to each tissue section scored. Two criteria were scored to document the pulmonary inflammation: peribronchial inflammation and perivascular inflammation. A value of 0 was adjudged when no inflammation was detectable, a value of 1 for occasional cuffing with inflammatory cells, a value of 2 when most bronchi or vessels were surrounded by a thin layer (1–5 cells) of inflammatory cells and a value of 3 when most bronchi or vessels were surrounded by a thick layer (>5

cells) of inflammatory cells. As 10–15 randomly selected tissue sections per mouse were scored, inflammation scores could be expressed as a mean value and could be compared between groups.

### Statistical analysis

Data were analysed with the statistical packet SPSS 6.1.2 (SPSS Inc., Chicago, IL, USA). All results are expressed as means ± SEM. ANOVA tests were applied to compare groups and post hoc analysis with Bonferroni's conservative correction was performed when ANOVA test were significant at  $P < 0.05$ .

## Results

### Bronchoalveolar lavage fluid

Repeated daily exposure of actively-immunized WT C57BL/6 mice to aerosolized OVA induced a significant increase in total cell numbers in the BALF (Table 1) when compared to the PBS-exposed WT animals. OVA exposure in these animals induced a significant eosinophilia, neutrophilia and lymphocytosis in the BALF. Repeated exposure of actively-immunized IL-10-deficient mice (IL-10<sup>-/-</sup>) also induced significantly higher cell numbers, eosinophilia, neutrophilia and lymphocytosis in the BALF when compared to the PBS-exposed IL-10<sup>-/-</sup> counterparts. Moreover, the increase in total cell numbers, eosinophilia and lymphocytosis observed in the OVA-exposed IL-10<sup>-/-</sup> mice was significantly higher than the increase observed in the OVA-exposed WT animals.

### Total and OVA-specific serum IgE

Total and OVA-specific serum IgE of the different animal groups are represented in Table 2. Exposure of actively-immunized WT mice to OVA induced a significant increase in total and allergen-specific serum IgE when compared to the PBS-exposed counterparts. Accordingly, OVA exposure in the immunized IL-10<sup>-/-</sup> mice resulted also in an increase

**Table 1.** Total cell numbers and cellular composition of BALF in actively sensitized wild-type and IL-10-deficient mice exposed to ovalbumin (OVA,  $n = 16$  and  $n = 13$ , respectively) aerosol or PBS ( $n = 9$  for both WT and IL10<sup>-/-</sup> mice). ANOVA \* $P < 0.05$  vs PBS exposed WT animals. † $P < 0.05$  vs PBS exposed IL-10<sup>-/-</sup> mice. ‡ $P < 0.05$  vs OVA challenged WT animals

|                                                  | WT<br>OVA/PBS  | WT<br>OVA/OVA       | IL-10 <sup>-/-</sup><br>OVA/PBS | IL-10 <sup>-/-</sup><br>OVA/OVA |
|--------------------------------------------------|----------------|---------------------|---------------------------------|---------------------------------|
| Total BALF cell<br>( $1 \times 10^5/\text{mL}$ ) | $1.9 \pm 0.2$  | $5.2 \pm 0.5^{*†}$  | $1.6 \pm 0.2$                   | $9.1 \pm 0.8^{*†‡}$             |
| Macrophage (%)                                   | $99.0 \pm 0.2$ | $69.6 \pm 2.6^{*†}$ | $99.0 \pm 0.2$                  | $31.7 \pm 3.6^{*†‡}$            |
| Lymphocyt (%)                                    | $0.3 \pm 0.1$  | $4.6 \pm 0.7^{*†}$  | $0.3 \pm 0.1$                   | $8.7 \pm 1.1^{*†‡}$             |
| Eosinophil (%)                                   | $0.0 \pm 0.0$  | $22.4 \pm 7.1^{*†}$ | $0.1 \pm 0.1$                   | $56.3 \pm 12.9^{*†‡}$           |
| Neutrophil (%)                                   | $0.6 \pm 0.1$  | $3.3 \pm 0.7^{*†}$  | $0.5 \pm 0.1$                   | $2.7 \pm 0.5^{*†}$              |

|                                | WT<br>OVA/PBS | WT<br>OVA/OVA                | IL-10 <sup>-/-</sup><br>OVA/PBS | IL-10 <sup>-/-</sup><br>OVA/OVA |
|--------------------------------|---------------|------------------------------|---------------------------------|---------------------------------|
| Total IgE ( $\mu\text{g/mL}$ ) | 3.3 $\pm$ 0.7 | 38.3 $\pm$ 4.8* <sup>†</sup> | 2.7 $\pm$ 0.7                   | 76.0 $\pm$ 9.4* <sup>†‡</sup>   |
| OVA-specific IgE (U/mL)        | 1.1 $\pm$ 0.4 | 40.7 $\pm$ 8.1* <sup>†</sup> | 0.8 $\pm$ 0.3                   | 53.2 $\pm$ 4.7* <sup>†</sup>    |

**Table 2.** Total and OVA-specific serum IgE in actively sensitized wild-type and IL-10-deficient (IL-10<sup>-/-</sup>) mice exposed to ovalbumin (OVA,  $n = 16$  and  $n = 13$ , respectively) aerosol or PBS ( $n = 9$  for both WT and IL-10<sup>-/-</sup> mice). ANOVA \* $P < 0.05$  vs PBS exposed WT animals. <sup>†</sup> $P < 0.05$  vs PBS-exposed IL-10<sup>-/-</sup> mice. <sup>‡</sup> $P < 0.05$  vs OVA-challenged WT animals

in total and OVA-specific serum IgE. Total IgE levels in the OVA-exposed IL-10<sup>-/-</sup> mice were significantly higher compared to the OVA-exposed WT mice whereas no significant difference was observed for allergen specific IgE between these two groups.

#### Airway responsiveness

Figure 1 shows the dose-response curves of airway responsiveness to carbachol. Sensitized WT or IL-10<sup>-/-</sup> mice exposed to OVA aerosol showed a significant hyperresponsiveness compared to the PBS-exposed WT animals. Airway responsiveness in PBS-exposed IL-10<sup>-/-</sup> mice was also significantly higher than in the PBS-exposed WT animals. Although OVA exposure induced a leftward shift of the dose-response curve in the IL-10<sup>-/-</sup> mice, the difference did not reach statistical significance after Bonferroni's conservative correction. Thus, the dose-response curves from the IL-10<sup>-/-</sup> mice, challenged with OVA or PBS and from the WT mice challenged with OVA did not differ ( $P > 0.05$ ), whereas these three groups were hyperresponsive relative to

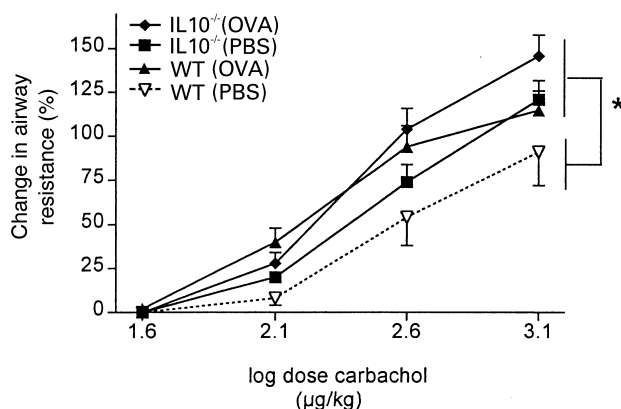


**Fig. 1.** Dose-response curves of the changes in airway resistance to increasing doses of carbachol. Wild-type mice (WT) exposed to ovalbumin (OVA,  $n = 16$ ) and IL-10-deficient (IL-10<sup>-/-</sup>) mice exposed to OVA ( $n = 13$ ) or PBS ( $n = 9$ ) aerosol are significantly hyperresponsive compared to WT mice exposed to PBS aerosol ( $n = 9$ ). ANOVA, \* $P < 0.05$ .

the PBS-challenged WT mice ( $P < 0.001$ ,  $P < 0.001$  and  $P = 0.002$ , respectively). These findings are also reflected by the PD<sub>50</sub>R<sub>L</sub> (Fig. 2). Whereas no significant differences in PD<sub>50</sub>R<sub>L</sub> were demonstrated between the IL-10<sup>-/-</sup> mice, challenged or not with OVA and WT mice challenged with OVA, the PD<sub>50</sub>R<sub>L</sub> of these animals were significantly lower than the PD<sub>50</sub>R<sub>L</sub> of the PBS-exposed WT animals (ANOVA,  $P < 0.007$ ).

#### Histopathology of the lungs

Figure 3 and Fig. 4 represent the histopathological scoring and photomicrographs of the pulmonary tissue in the different groups of mice. Histological evaluation of the airways from the WT (Fig. 4, panel A) and IL-10<sup>-/-</sup> (Fig. 4, panel C) mice exposed to PBS aerosol showed normal lung histology in both groups. Sensitization and subsequent

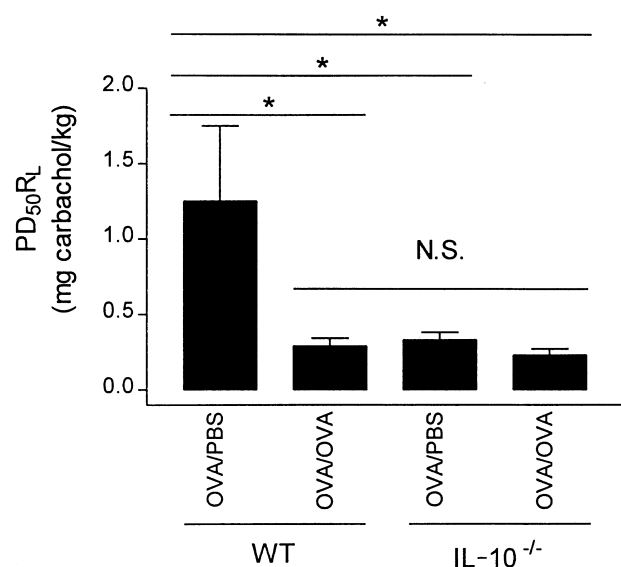


**Fig. 2.** PD<sub>50</sub>R<sub>L</sub> is significantly higher in wild-type (WT) animals exposed to PBS ( $n = 9$ ) when compared to WT animals exposed to ovalbumin (OVA,  $n = 16$ ) or when compared to the IL-10-deficient mice (IL-10<sup>-/-</sup>) exposed to OVA ( $n = 13$ ) or PBS ( $n = 9$ ). ANOVA, \* $P < 0.05$ . N.S. not significant.

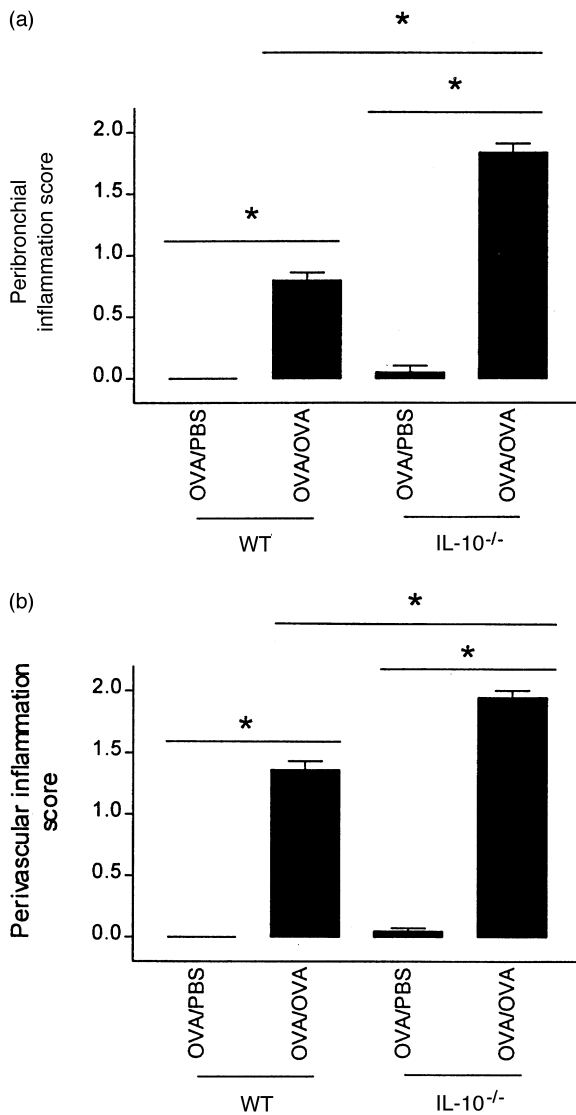


**Fig. 3.** Peribronchial and perivascular inflammation scores in actively sensitized wild-type (WT) and IL-10-deficient (IL-10<sup>-/-</sup>) mice exposed to ovalbumin (OVA) aerosol or PBS. ANOVA, \* $P < 0.05$  for different subgroups.

exposure to OVA resulted in a significant peribronchial and perivascular eosinophilic inflammation both in the WT (Fig. 4, panel B) and IL-10<sup>-/-</sup> (Fig. 4, panel D) animals when compared to the PBS-exposed counterparts. Moreover, the inflammation observed in the OVA-exposed IL-10<sup>-/-</sup> mice was significantly more pronounced than the inflammation observed in the OVA-exposed WT mice (ANOVA,  $P < 0.0001$ ). The infiltrations observed in the peribronchial areas consisted mainly of lymphocytes, macrophages and eosinophils. The alveolar septa were free of inflammation.

#### Analysis of cytokines in BALF

Concentrations of IFN  $\gamma$  in the BALF of OVA-exposed animals were significantly (ANOVA,  $P < 0.0001$ ) lower ( $3.3 \pm 0.2$  and  $3.8 \pm 0.3$  pg/mL for WT and IL-10<sup>-/-</sup> mice, respectively) when compared to the PBS-exposed animals ( $6.7 \pm 0.5$  and  $6.0 \pm 0.7$  pg/mL for WT and IL-10<sup>-/-</sup> mice, respectively). The IL-5 concentrations in the BALF were significantly (ANOVA,  $P < 0.0001$ ) higher in the OVA-exposed animals ( $781 \pm 130$  and  $261 \pm 41$  fg/mL for WT and IL-10<sup>-/-</sup> mice, respectively) when compared to the PBS-exposed animals ( $44 \pm 14$  and  $123 \pm 30$  fg/mL for WT and IL-10<sup>-/-</sup> mice, respectively). Moreover, IL-5 concentrations were significantly higher in BALF from the OVA-exposed WT animals compared to the OVA exposed IL-10<sup>-/-</sup> mice. IL-4 concentrations in the BALF were lower (ANOVA,  $P = 0.001$ ) in the OVA-exposed WT animals ( $21 \pm 2$  pg/mL) when compared to the PBS-exposed WT ( $46 \pm 5$  pg/mL), the PBS-exposed IL-10<sup>-/-</sup> ( $49 \pm 7$  pg/mL) and the OVA-exposed IL-10<sup>-/-</sup> ( $37 \pm 7$  pg/mL) mice. IL-13 was detectable in 56% (9/16) of the OVA-exposed WT animals and 50% (3/6) of the OVA-exposed IL-10<sup>-/-</sup> animals ( $3.2 \pm 0.6$  and  $1.3 \pm 0.1$  pg/mL, respectively), while we could not detect IL-13 in the BALF of the PBS-exposed WT or IL-10<sup>-/-</sup> animals.

#### Discussion

IL-10 deficiency leads to an enhanced eosinophilic airway inflammation during allergen exposure in sensitized mice and is associated with an increase in baseline airway responsiveness. Sensitized and allergen-exposed IL-10-deficient mice have also higher total serum IgE than the WT controls.

The suppressive activity of endogenous IL-10 in allergen-induced airway inflammation is in agreement with earlier findings from *in vitro* experiments [16,17], from experiments in which exogenous rIL-10 was administered in murine asthma models [18,19] and from clinical data showing that alveolar macrophages from asthmatic patients produce significantly lower amounts of IL-10 compared to non asthmatic healthy volunteers [11] while a genotype, associated with low IL-10 production, was found in patients with severe asthma [10]. The anti-inflammatory role of IL-10 was not mediated through IFN  $\gamma$ , IL-4 or IL-5 as measured in the BALF. Since the OVA-exposed WT mice had even more IL-5 in the BALF compared to the OVA-exposed IL-10<sup>-/-</sup> mice, it is unlikely that under the present experimental conditions the BALF concentrations of IL-5 are important in determining the severity of airway inflammation and BALF eosinophilia. Moreover, it was demonstrated that rather the circulating but not the local lung IL-5 is required for the development of antigen-induced airway eosinophilia [20] suggesting that BALF IL-5 concentrations

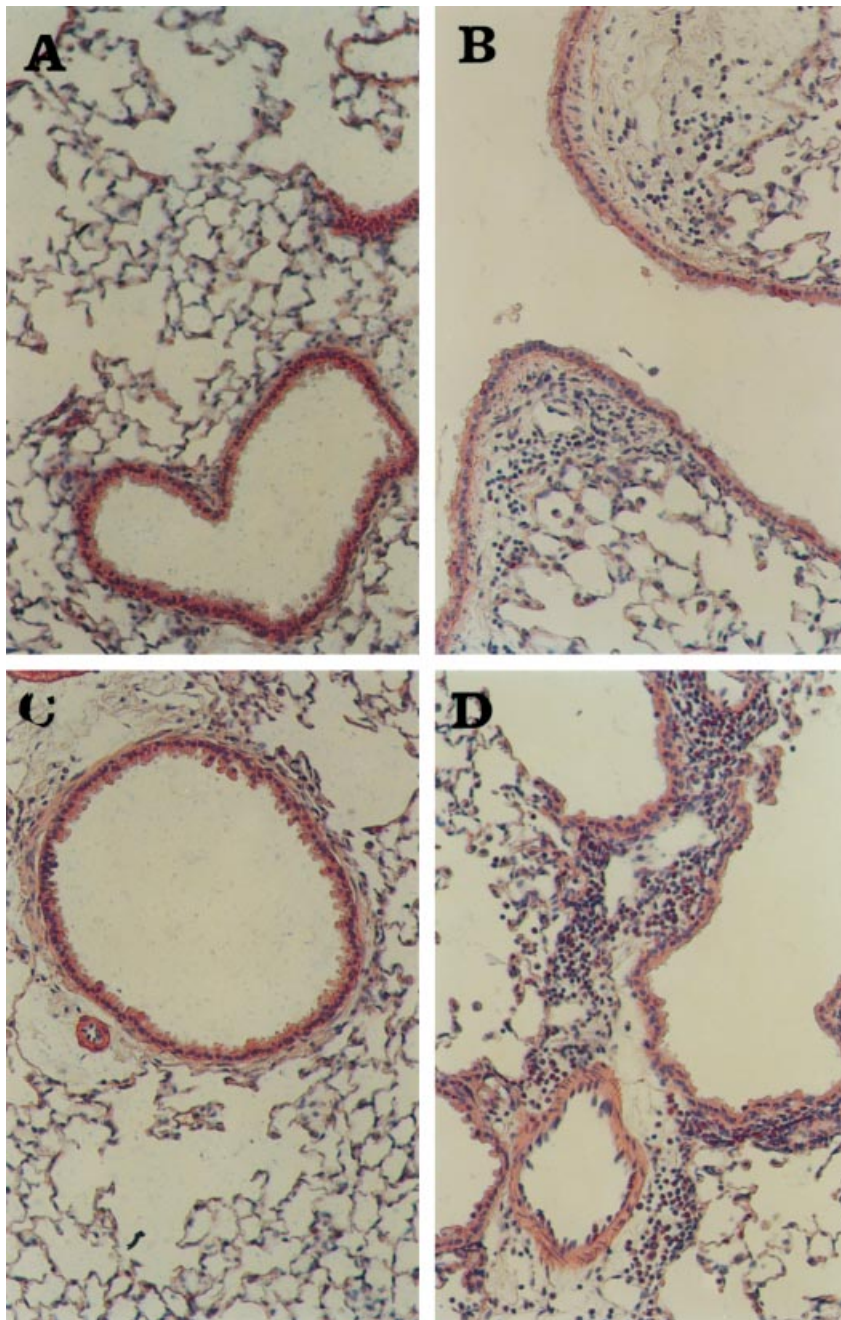


**Fig. 4.** Photomicrographs depicting the pulmonary tissue of the four groups of mice (magnification  $\times 200$ ). Wild-type mice exposed to PBS (panel A) or to OVA (panel B) and IL-10<sup>-/-</sup> mice exposed to PBS (panel C) or to OVA (panel D).

are less relevant. Our findings of lower IL-5 in the BALF of the IL10<sup>-/-</sup> mice differ from *in vitro* observations [21,22]. In a murine model of bronchopulmonary aspergillosis [21], it was shown that lung cells from IL10<sup>-/-</sup> mice produced more IL-5 as well as IL-4 and IFN  $\gamma$  than WT animals after stimulation with anti-CD3 antibodies. In addition, it was shown that exogenous rmIL-10 blocked IL-5 generation by sensitized peritoneal cells [22]. However, findings from *in vitro* studies or studies in which a single Ag challenge is given do not necessarily reflect the effect of endogenously

produced interleukins as it occurs in our model with a 7-day repeated allergen exposure scheme. Since IFN  $\gamma$  was equally suppressed in the OVA-exposed IL-10<sup>-/-</sup> and WT mice compared to the PBS-exposed IL-10<sup>-/-</sup> or WT mice, the involvement of this cytokine is unlikely to be important in the anti-inflammatory effects of IL-10. We must therefore conclude that the increased allergen-induced inflammation that we observed in the IL10<sup>-/-</sup> mice is not mediated through an enhanced production of pro-inflammatory cytokines or a decreased production of IFN  $\gamma$  as measured in the BALF.

The findings with BALF cytokines should however, be interpreted with caution since it is largely unknown whether differences in BALF cytokines reflect what is happening in the pulmonary tissue. There are a number of factors that could interfere with the BALF cytokine concentration measurements and that therefore could make BALF cytokine analysis less relevant. Some of these factors were controlled (e.g. the presence of more proteases in the groups with more inflammation was handled by adding protease inhibitors to the lavage fluid) and others could not be controlled (e.g. presence of molecules that could interfere with the ELISA such as haptoglobin, or differences in surface or soluble cytokine receptors between the groups). A differential effect depending on the type of cytokine measured is also possible. That could explain why the values of IFN  $\gamma$  and IL-13 were as expected whereas the values of IL-4 and IL-5 were not. Especially IL-5 turned out to be a very difficult cytokine for detection, and even despite the concentration procedure, the measured values were very low. Although a direct effect of IL-10 is an attractive hypothesis based on the measured BALF cytokines, we cannot fully exclude the possibility of an effect through cytokines present in the pulmonary tissue.

Airway hyperresponsiveness was observed in the OVA-exposed WT and IL-10<sup>-/-</sup> animals when compared to the PBS-exposed WT counterparts. Moreover, in our study, the PBS-exposed IL-10<sup>-/-</sup> mice showed a higher baseline airway responsiveness compared with the PBS-exposed WT animals. It is theoretically possible that the airways of these IL-10<sup>-/-</sup> mice are more sensitive to environmental stimuli because of the enhanced inflammatory reaction to a variety of exogenous stimuli (for example food or bedding antigens in the intestines). Especially IL-10<sup>-/-</sup>  $\times$  BALB/c mice are extremely prone to develop spontaneously inflammation in their intestines with colorectal prolapse [23] whereas the IL-10<sup>-/-</sup>  $\times$  C57Bl/6 mutants do not develop inflammatory bowel diseases when kept under specific pathogen-free conditions [24]. Although we took the latter mice for our experiments when they were 6–8 weeks and under strictly pathogen-free conditions and although their intestines and airways were free of inflammation at the time of pulmonary function, it remains possible that the inevitable environmental exposure might have contributed to the higher baseline airway responsiveness of the PBS-exposed IL-10<sup>-/-</sup> animals compared with the WT animals. How exactly the absence of endogenously-produced IL-10 augments the airway responsiveness warrants further research. There was no morphological evidence of airway inflammation in the IL-10<sup>-/-</sup> mice exposed to PBS and there were no arguments suggesting an effect through the BALF cytokines IL-4, IL-5, IL-13 and IFN  $\gamma$ . A direct inhibitory effect of endogenous IL-10 on the airway smooth muscle cells or indirect effects through the stimulation or inhibition of unknown mediators of the airway tone are distinct

possibilities. Since no significant difference in airway responsiveness was observed between the OVA-exposed WT and IL-10<sup>-/-</sup> mice despite the presence of more eosinophils in the latter group, and since PBS-exposed IL-10<sup>-/-</sup> mice had increased airway reactivity in the absence of airway eosinophilia, one could question the role of the eosinophils in the induction of airway hyperresponsiveness. There is indeed increasing evidence that airway hyperresponsiveness in sensitized subjects can be induced by allergic sensitization without involvement of eosinophils [25–31]. Another possibility is that the OVA-exposed IL-10<sup>-/-</sup> mice were unable to mount higher airway reactivity because of mechanical restrictions. However, since the average increase in airway resistance was only 145% when 1200  $\mu$ g carbachol/kg body weight was injected, we believe that the maximum was not reached. Earlier experiments demonstrated that mice are able to increase their airway resistance to over 200%.

Next to the presence of more eosinophils, the IL-10<sup>-/-</sup> mice exposed to OVA had higher total serum IgE levels whereas OVA-specific serum IgE was comparable to the levels found in the WT mice. The influence of IL-10 on IgE synthesis has been evaluated by other investigators. It was demonstrated *in vitro* that IL-10, when present from the beginning of the culture, decreases IL-4-induced IgE production [32]. On the other hand, IL-10 together with IL-4 is capable of enhancing allergen-specific IgE synthesis in unprimed B cells exposed to allergen, but does not further stimulate the spontaneous secretion of allergen-specific IgE from antigen-primed B cells [33]. Taken together, these findings are in agreement with our results where comparable levels of specific IgE in the allergen-challenged WT and IL-10<sup>-/-</sup> groups were found. Surprisingly, we observed significantly lower IL-4 levels in the BALF of the OVA-exposed WT animals compared to their PBS-exposed littermates. IL-4 is the key cytokine in the initiation of IgE synthesis both in mouse and man [34,35], but little is known about the regulation of the IL-4 production during the secondary allergen challenge. Findings that IL-4 concentrations in BALF are lower in saline-exposed animals and that BALF IL-4 does not correlate with the serum IgE levels were reported by others [31]. This implicates that IL-4 might be less important in the late phase of the IgE production. It was demonstrated that in atopy persistent stimulation of CD4<sup>+</sup>CD45RO<sup>+</sup> memory/effector T cells is able to provide contact-dependent activation signals to B cells, and that these cells induce IgE production largely via IL-13 rather than via IL-4 [36]. This is in agreement with our findings since IL-13 was detectable only in the animals which were exposed to OVA and which produced serum IgE.

In conclusion, these findings suggest that IL-10 plays an important role in the control of the airway inflammation

and altered responsiveness in an *in vivo* mouse model of allergy.

### Acknowledgements

Dr K. Tournoy is granted by the Fund for Scientific Research – Flanders (FWO-Vlaanderen). The authors wish to thank E. Castrique, C. Snauwaert, A. Neesen, I. De Borle, K. De Saedeleer and M. Mouton for their excellent technical assistance.

### References

- 1 Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989; 139:806–17.
- 2 Synek M, Beasley R, Frew AJ, Goulding D, Holloway L, Lampe FC, Roche WR, Holgate ST. Cellular infiltration of the airways in asthma of varying severity. *Am J Respir Crit Care Med* 1996; 154:224–30.
- 3 Cho SH, Seo JY, Choi DC, Yoon HJ, Cho YJ, Min KU, Lee GK, Seo JW, Kim YY. Pathological changes according to the severity of asthma. *Clin Exp Allergy* 1996; 26:1210–9.
- 4 Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326:298–304.
- 5 Bousquet J, Chanez P, Lacoste J, Barnéon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J, Godard P, Michel F. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–9.
- 6 Drazen JM, Arm JP, Austen KF. Sorting out the cytokines of asthma. *J Exp Med* 1996; 183:1–5.
- 7 Swain SL. Who does the polarizing? *Eur J Immunol* 1995; 5:849–51.
- 8 Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J Exp Med* 1989; 170:2081–95.
- 9 Borish L. IL-10: evolving concepts. *J Allergy Clin Immunol* 1998; 101:293–7.
- 10 Lim S, Crawley E, Woo P, Barnes PJ. Haplotype associated with low interleukin-10 production in patients with severe asthma. *Lancet* 1998; 352:113.
- 11 John M, Lim S, Seybold J, Jose P, Robichaud A, O'Connor B, Barnes PJ, Chung KF. Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1 $\alpha$ , granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. *Am J Respir Crit Care Med* 1998; 157:256–62.
- 12 Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci* 1998; 94:557–72.
- 13 Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993; 75:263–74.
- 14 Kips JC, Brusselle GJ, Joos GF, Peleman RA, Tavernier JH, Devos RR, Pauwels RA. Interleukin-12 inhibits antigen-induced airway hyperresponsiveness in mice. *Am J Respir Crit Care Med* 1996; 153:535–9.
- 15 Curtis JL, Byrd PK, Warnock ML, Kaltreider HB. Requirement of CD4-positive T cells for cellular recruitment to the lungs of mice in response to a particulate intratracheal antigen. *J Clin Invest* 1991; 88:1244–54.
- 16 Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J Immunol* 1993; 150:353–60.
- 17 Enk AH, Angeloni VL, Udey MC, Katz SI. Inhibition of Langerhans cell antigen-presenting function by IL-10. A role for IL-10 in induction of tolerance. *J Immunol* 1993; 151:2390–8.
- 18 Zuany-Amorim C, Haile S, Leduc D, Dumarey C, Huerre M, Vargaftig BB, Pretolani M. Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. *J Clin Invest* 1995; 95:2644–51.
- 19 Van Scott MR, Bradfield J, Metzger WJ, Welch CA, Knox C, Sigounas A, Kukoly C, Sur S. IL-10 inhibits ragweed-induced lung inflammation, but increases airway resistance in allergic mice. *Am J Respir Crit Care Med* 1997; 155:A734.
- 20 Wang J, Palmer K, Lotvall J, Milan S, Lei XF, Matthaei KI, Gauldie J, Inman MD, Jordana M, Xing Z. Circulating, but not local lung, IL-5 is required for the development of antigen-induced airways eosinophilia. *J Clin Invest* 1998; 102:1132–41.
- 21 Grunig G, Corry DB, Leach MW, Seymour BWP, Kurup VP, Rennick DM. Interleukin-10 is a natural suppressor of cytokine production and inflammation in a murine model of allergic bronchopulmonary aspergillosis. *J Exp Med* 1997; 185:1089–99.
- 22 Zuany-Amorim C, Creminon C, Nevers MC, Nahori M, Vargaftig BB, Pretolani M. Modulation by IL-10 of antigen-induced IL-5 generation, and CD4<sup>+</sup> lymphocyte and eosinophil infiltration into the mouse peritoneal cavity. *J Immunol* 1996; 157:377–84.
- 23 Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Holland G, Thompson Snipes L, Leach MW, Rennick D. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4<sup>+</sup> TH1-like responses. *J Clin Invest* 1996; 98:1010–20.
- 24 Bristol IJ, Mahler M, Leiter EH, Sundberg JP. IL10<sup>tm1Cgn</sup>, an interleukin-10 gene targeted mutation. *Jax Notes* 1997; 471.
- 25 Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener Kronish JP, Locksley RM. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 1996; 183:109–17.
- 26 Lefort J, Bachelet CM, Leduc D, Vargaftig BB. Effect of antigen provocation of IL-5 transgenic mice on eosinophil mobilization and bronchial hyperresponsiveness. *J Allergy Clin Immunol* 1996; 97:788–99.
- 27 Matsubara S, Fushimi K, Ogawa K, Kikkawa H, Nakata A, Kameda R, Kikuchi M, Naito K, Ikezawa K. Inhibition of pulmonary eosinophilia does not necessarily prevent the airway

- hyperresponsiveness induced by Sephadex beads. *Int Arch Allergy Immunol* 1998; 116:67–75.
- 28 Hogan SP, Mattheai KI, Young JM, Koskinen A, Young IG, Foster PS. A novel T cell-regulated mechanism modulating allergen-induced airways hyperreactivity in BALB/c mice independently of IL-4 and IL-5. *J Immunol* 1998; 161:1501–9.
- 29 Hessel EM, Van Oosterhout AJ, Van Ark I, Van Esch B, Hofman G, Van Loveren H, Savelkoul FJ, Nijkamp FP. Development of airway hyperresponsiveness is dependent on interferon- and independent of eosinophil infiltration. *Am J Respir Cell Mol Biol* 1997; 16:325–34.
- 30 Coyle AJ, Kohler G, Tsuyuki S, Brombacher F, Kopf M. Eosinophils are not required to induce airway hyperresponsiveness after nematode infection. *Eur J Immunol* 1998; 28: 2640–7.
- 31 Zuany-Amorim C, Ruffie C, Haile S, Vargaftig BB, Pereira P, Pretolani M. Requirement for gamma delta T cells in allergic airway inflammation. *Science* 1998; 280:1265–7.
- 32 Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* 1998; 160:3555–61.
- 33 Ohmori H, Kanda T, Takai T, Hikida M. Induction of antigen-specific IgE response in murine lymphocytes by IL-10. *Immunol Lett* 1995; 47:127–32.
- 34 Snapper CM, Paul WE. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 1987; 236:944–7.
- 35 Pene J, Rousset F, Briere F, Chretien I, Bonnefoy JY, Spits H, Yokota T, Arai N, Arai K, Banchereau J *et al.* IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. *Proc Natl Acad Sci U S A* 1988; 85:6880–4.
- 36 Levy F, Kristofic C, HeusSeries C, Brinkmann V. Role of IL-13 in CD4 T cell-dependent IgE production in atopy. *Int Arch Allergy Immunol* 1997; 112:49–58.