

## ORIGINAL ARTICLE Experimental Models of Allergic Disease

# Airway inflammation evaluated in a human nasal lipopolysaccharide challenge model by investigating the effect of a CXCR2 inhibitor

R. Virtala<sup>1</sup>, A.-K. Ekman<sup>2</sup>, L. Jansson<sup>1</sup>, U. Westin<sup>3</sup> and L. O. Cardell<sup>2</sup>

<sup>1</sup>Astra Zeneca R&D, Lund, Sweden, <sup>2</sup>Division of ENT Diseases, CLINTEC, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden and

<sup>3</sup>Department of Otorhinolaryngology, Skåne University Hospital, Lund University, Malmö, Sweden

## Clinical & Experimental Allergy

### Summary

**Background** The existence of a link between inflammation in upper and lower airways is well established. It may therefore be assumed that the nose could be used to study inflammatory events in the lower airways.

**Objective** This study aimed to evaluate a lipopolysaccharide (LPS) nasal challenge model by investigating the effect of the CXCR2 inhibitor AZD8309 on neutrophilic inflammation. **Methods** A total of 18 healthy volunteers were randomized in a placebo-controlled, double-blind, cross-over study. AZD8309 or placebo was dosed for 3 days. Subjects were challenged nasally with LPS (50 µg/nostril), and nasal lavage was performed 6 and 24 h later. Leucocytes, neutrophils and inflammatory mediators were assessed in the lavage fluid. The outcome was compared with data from analogous experiments performed in a model of inhaled LPS followed by induced sputum. This trial was registered in the Current Controlled Trials register (ISRCTN trial number: ISRCTN46666382).

**Results** The leucocytes in nasal lavage consisted to 99% of neutrophils on average. Treatment with AZD8309 reduced the leucocyte count to 48% of placebo 6 h after the LPS challenge. There was also a reduction in LTB<sub>4</sub> levels to 45% of placebo after 6 h and in the neutrophil elastase activity after 24 h. No major adverse events were seen with either AZD8309 or placebo. The nasal LPS model induced only minimal local irritation and no signs of systemic inflammation.

**Conclusions and Clinical Relevance** LPS-induced neutrophil recruitment was reduced by inhibition of CXCR2. This outcome mimicked the response previously seen in a lower airway LPS model. Hence, the nasal model offers a convenient and well-tolerated alternative for pharmacological evaluation of anti-inflammatory drugs affecting neutrophilic migration and activity.

**Keywords** airway, endotoxin, inflammation, innate immunity, lavage, nasal, neutrophil

*Submitted 18 February 2011; revised 07 October 2011; accepted 13 October 2011*

### Correspondence:

Lars Olaf Cardell, Head of the ENT Division, Department of Clinical Sciences, Intervention and Technology, Karolinska Institutet, Karolinska University Hospital, S-141 86 Stockholm, Sweden.  
E-mail: lars-olaf.cardell@ki.se

### Introduction

The link between inflammation in the upper and the lower airways is well established, as seen with allergic rhinitis and asthma [1]. The nose and lungs show similarities both in terms of cellularity and inflammatory responses [2, 3]. The inhaled lipopolysaccharide (LPS) challenge model has been extensively used to investigate mechanisms in inflammatory respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) [4, 5]. This method is based on the activation of Toll-like receptor (TLR) 4 by LPS derived from

gram-negative bacteria [6–8] and the subsequent induction of a local inflammatory response.

The LPS-induced cell egress in respiratory secretions is to a large extent composed of neutrophils [9, 10], similar to what is seen in COPD [4]. Therefore, the inhaled LPS challenge model is used also in drug development as a disease model both in animals and humans [11–13]. Such studies have, however, shown that the rodent LPS models do not uniformly translate to humans [14–16]. Because of this, there is a need to develop and use the human LPS challenge model early in drug development. Ideally, the model should be

reliable, easy to apply and without discomfort for the study participant. Such a model may facilitate the study of drugs for treatment of diseases characterized by neutrophil inflammation, for example, COPD.

To investigate the LPS nasal challenge method, we have in the present study evaluated the effects of the CXCR2 antagonist AZD8309 on neutrophil inflammation in nasal lavage after nasal LPS challenge.

## Methods

### Study design

The study was a randomized, double-blind, placebo-controlled, two-way cross-over study in healthy volunteers to evaluate whether AZD8309 inhibits the increase of neutrophils in nasal lavage after LPS challenge. The study comprised of six visits: a screening visit (visit 1), four treatment visits (visits 2, 3, 4 and 5) and a follow-up visit (visit 6). At visit 2, the subjects were randomized to receive either AZD8309 or placebo. AZD8309 was administered as 300 mg of an oral solution of 30 mg/g. Dosing was done twice daily for 2 days. Assessment of leucocytes and CRP in blood was performed before the first dose and 7-h post-dose. On the third day, visit 3 started and the subjects were dosed once again. One hour after dosing, nasal challenges with LPS were performed. Samples of both nasal lavage and blood were taken pre-dose and repeatedly after the LPS challenge. The subjects took one further dose approximately 11 h after the LPS challenge. The following morning (24-h post-LPS), samples of nasal lavage and blood were collected once again. Symptoms were evaluated by the subjects as previously described [10].

After a wash-out period of at least 3 weeks, the subjects returned for visits 4 and 5. They underwent the same procedure as at visits 2 and 3, but those subjects that received AZD8309 on the previous visits now received placebo and *vice versa*. This trial was registered in the Current Controlled Trials register (ISRCTN trial number: ISRCTN46666382). The study design is illustrated in Fig. 1. The study protocol was approved by the Ethics Committee Lund University.

### LPS challenge

At visits 3 and 5, 1 h after the morning dose of AZD8309 or placebo, the subjects were sprayed with 100 µL of a 0.50 µg/µL LPS solution (*Escherichia coli* 026: B6, purchased from Sigma Aldrich, Stockholm, Sweden) in each nasal cavity, resulting in a challenge dose of totally 100 µg LPS. The dose 100 µg was chosen as this dose in an earlier study was found not to induce systemic effects [10]. The LPS was manufactured in Israel by fermentation and supplied as a lyophilized

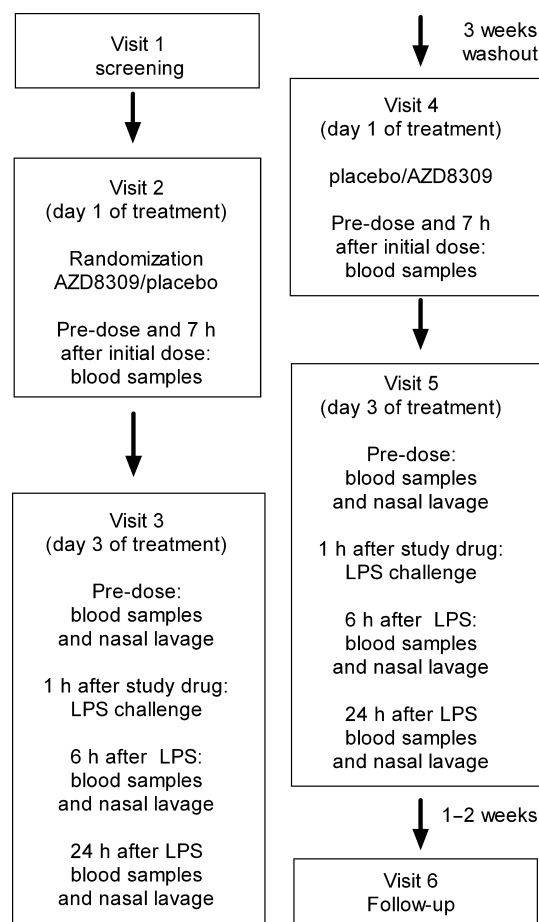


Fig. 1. Data flow chart of the study design.

powder in 1 mg ampoules sterilized by gamma radiation. A specific batch of LPS was chosen, and this batch was used throughout the study. The concentration of this batch, before and after reconstitution with saline and storage, was verified by Limulus Amebocyte Lysate analysis at AstraZeneca R&D Södertälje, Sweden. The lyophilized LPS powder was reconstituted with saline at AstraZeneca R&D Lund, or at the study site, and was stored at  $-20^{\circ}\text{C}$  (or below) until use, and 6 and 24 h after LPS challenge, nasal lavage was performed.

### Nasal lavage

Nasal lavage fluids were obtained as previously described [17]. After clearing excess mucus by forceful exsufflation, sterile saline solution (0.9% NaCl) of room temperature was aerosolized into the nostrils. The fluid was collected as it passively returned. The procedure was repeated alternating the nostrils until 7 mL of the lavage fluid was recovered. The lavage fluid was centrifuged for 10 min at 1334 g at  $4^{\circ}\text{C}$ . The supernatant was separated from the pellet and stored at  $-70^{\circ}\text{C}$  until analysed for mediators. The cell pellet was kept for total cell counts and preparation of slides for determination of differential counts.

### *Leucocytes and C-Reactive protein in peripheral blood*

For evaluation of systemic effects of AZD8309 treatment, total leucocyte counts and differential counts were determined in whole blood by using ethylene diamine tetraacetic acid tubes and an Automatic cell counter. High-sensitivity C-Reactive protein (CRP) was measured in serum using serum-separating tubes and an immunoturbidimetric assay.

### *Leucocytes in nasal lavage*

The cell pellet was redispersed in saline, a slide smear was prepared for differential count, and the total cell counts of live leucocytes were counted in a Bürker chamber. Differential counts were estimated on the prepared slides after May-Grünwald and Giemsa staining. Leucocyte cell morphology was assessed using a microscope, and the percentage of neutrophils, eosinophils, lymphocytes and macrophages was determined out of a total of 100 leucocytes.

### *Analysis of mediators and neutrophil elastase activity in nasal lavage*

GRO- $\alpha$  and IL-8 in nasal lavage were measured using Quantikine<sup>®</sup> sandwich enzyme immunoassays Human CXCL1/GRO- $\alpha$  Immunoassay (Cat. No. DGR00) and Human CXCL8/IL-8 Immunoassay (Cat. No. D8000C) from R&D Systems, Inc., Minneapolis, MN, USA. LTB<sub>4</sub> was measured using the competitive enzyme immunoassay Leukotriene B<sub>4</sub> EIA Kit (Cat. No. D8000C, Cayman Chemical Company, Ann Arbor, MI, USA). Neutrophil elastase activity was determined using a fluorogenic peptide substrate, MeOSuc-Ala-Ala-Pro-Val-7-amido-4-methylcoumarin (Cat. No. 324740, VWR International AB, Stockholm, Sweden). Five microlitres of 1 mM substrate in buffer [0.5 M NaCl (Cat. No. 16404-1, VWR International AB), 0.1 M Tris-HCl, pH 7.5 (Cat. No. T-5941, Sigma Aldrich) and 0.01% (v/v) TritonX-100 (Cat. No. 234729, Sigma Aldrich)] were added to the samples, and fluorescence was measured in 10-min intervals for 240 min using a Perkin Elmer EnVision 2102 instrument at excitation 380 nm and emission 460 nm. The rate of substrate turnover was calculated by linear regression. AMC (7-amino-4-methylcoumarin) and purified human neutrophil elastase (Cat. No. 08440 and 324681, VWR International AB) were used as standards.

### *Statistical and analytical methods*

The statistical analysis was conducted using Gauss from Aptech Systems Inc. (kernel revision 6.0.57) and the Riemann Library (v.2.4.1; Black Diamond, WA, USA).

Treatment effects were analysed under a multiplicative ANOVA model with factors treatment, period and subject. Results were expressed as the ratio of AZD8309 treatment to placebo treatment, using geometric means to account for the log-normal distribution of data. All hypothesis testing was done using two-sided alternative hypotheses. *P*-values less than 0.05 were considered statistically significant.

## **Results**

Of 18 enrolled subjects, 16 completed the study. Symptoms of the AZD8309 treatment compared with placebo were overall mild. Of the 16 subjects, two reported mild gastrointestinal symptoms, otherwise adverse effects were slight headache or pyrexia, and reported by only a few participants. Nasal LPS challenge caused minimal discomfort, and no systemic reactions in the placebo group either clinically (chills or malaise) or observed with laboratory measurements (leucocytosis, change in differential count or increased CRP) were found (data not shown).

In the nasal lavage fluid the total leucocyte count was measured in all samples, but the differential counts could only be evaluated in 11 subjects. The leucocyte population consisted to 99% of neutrophils on average (data not shown).

Before the LPS challenge, no difference in the number of leucocytes [placebo: 4.04 (min-max: 0.7–15)  $\times 10^4$  cells/mL vs. AZD8309: 1.60 (min-max: 0.3–7.3)  $\times 10^4$  cells/mL], or neutrophils [placebo: 4.27 (min-max: 1.96–7.58)  $\times 10^4$  cells/mL vs. AZD8309: 1.67 (min-max: 1–4.7)  $\times 10^4$  cells/mL] in nasal lavage were seen between the two study groups (data not shown).

Treatment with AZD8309 markedly reduced the leucocyte count to 48% of the placebo value 6 h after the LPS challenge (*P* = 0.001). The reduction in leucocyte numbers was still visible after 24 h (*P* = 0.018; Figs 2 and 3; Tables 1 and 2).

A pronounced neutrophil egress was visible 6 h after challenge, and fewer neutrophils were found in the nasal lavage fluid in the AZD8309 group than in the placebo group (*P* = 0.013). The levels of neutrophils in the AZD8309 treated group comprised 57% of the placebo levels (Fig. 2; Table 1).

Treatment with AZD8309 caused a strong trend for reduced neutrophil elastase activity compared with placebo, 6 h after challenge, with a statistically significant reduction compared with placebo after 24 h (*P* = 0.009; Figs 2 and 3; Tables 1 and 2). The levels of LTB<sub>4</sub> in lavage fluid was markedly reduced at 6 h after challenge in the AZD8309-treated participants compared with the placebo group (*P* < 0.001), a reduction which was sustained at 24 h (*P* = 0.045; Figs 2 and 3; Tables 1 and 2). Furthermore, there was a trend for a

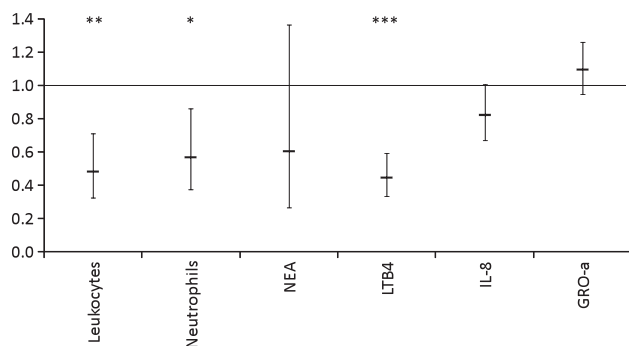


Fig. 2. Treatment ratios for nasal lavage data 6 h after lipopolysaccharide challenge. Solid bars indicate ratios of AZD8309 to placebo, and error bars indicate 95% confidence intervals. Statistically significant results are indicated with \*, \*\* and \*\*\* for *P*-values below 0.05, 0.01 and 0.001, respectively. NEA, neutrophil elastase activity.

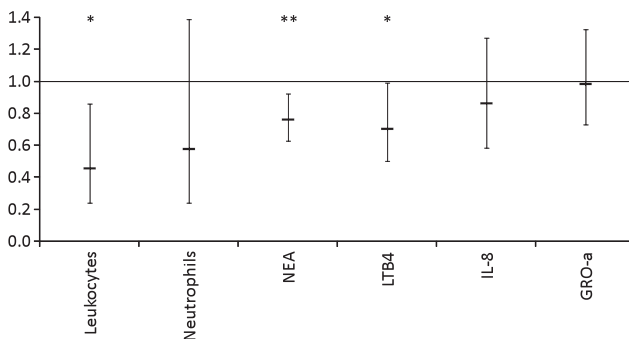


Fig. 3. Treatment ratios for nasal lavage data 24 h after lipopolysaccharide challenge. Solid bars indicate ratios of AZD8309 to placebo, and error bars indicate 95% confidence intervals. Statistically significant results are indicated with \* and \*\* for *P*-values below 0.05 and 0.01, respectively. NEA, neutrophil elastase activity.

reduction of IL-8 in subjects treated with AZD8309 compared with subjects who received placebo, 6 h after LPS challenge ( $P = 0.058$ ), which was abolished 24 h after challenge (Figs 2 and 3; Tables 1 and 2). The levels of GRO- $\alpha$  were similar after treatment with AZD8309 compared with placebo at all measured time-points (Figs 2 and 3; Tables 1 and 2).

## Discussion

In contrast to the inhaled bronchial LPS challenge model, nasal application of LPS has been less frequently used as a research tool in the evaluation of respiratory inflammatory disease. The present study evaluates the LPS nasal model in a study designed to investigate neutrophils, likely egressed from blood, in respiratory secretions after the treatment with AZD8309. This compound, which has been under development in AstraZeneca R&D, Lund, Sweden, is a CXCR2 inhibitor (a chemokine receptor antagonist). The CXCR2 receptor is expressed on neutrophils [18], and an inhibitor is expected to decrease the chemotactic effects induced by an inflammatory stimulus via CXC receptor ligands such as IL-8 [19]. A previous study with AZD8309 designed to test the effects in the inhaled LPS challenge model showed attenuation of the egress of neutrophils to induced sputum [20]. Thus, the present study enables a comparison of both results and feasibility between the two different challenge models.

The model of inhaled LPS followed by induced sputum demonstrated a reduction in the total number of leucocytes, the amount of neutrophils, and in the levels of neutrophil elastase activity in response to AZD8309 treatment. It also displayed a trend for a reduction of

Table 1. Statistical comparison of nasal lavage data 6 h after lipopolysaccharide challenge

| Variable                         | Treatment | <i>n</i> | Gmean | CV (%) | Min | Max    | Analysis of ratio* |            |                 |
|----------------------------------|-----------|----------|-------|--------|-----|--------|--------------------|------------|-----------------|
|                                  |           |          |       |        |     |        | Est.               | 95% CL     | <i>P</i> -value |
| Leucocytes ( $10^4/\text{mL}$ )  | AZD8309   | 16       | 5.0   | 106    | 1.0 | 19     | 0.48               | 0.32, 0.71 | 0.001           |
|                                  | Placebo   | 16       | 10    | 97     | 1.7 | 35     |                    |            |                 |
| Neutrophils ( $10^4/\text{mL}$ ) | AZD8309   | 11       | 6.8   | 78     | 2.0 | 19     | 0.57               | 0.28, 0.86 | 0.013           |
|                                  | Placebo   | 11       | 12    | 75     | 3.7 | 33     |                    |            |                 |
| NEA ( $10^3$ fluor units)        | AZD8309   | 16       | 65    | 108    | 30  | 647    | 0.60               | 0.27, 1.4  | 0.21            |
|                                  | Placebo   | 16       | 108   | 290    | 30  | 10 200 |                    |            |                 |
| LTB4 (pg/mL)                     | AZD8309   | 16       | 185   | 121    | 50  | 1063   | 0.45               | 0.34, 0.59 | <0.001          |
|                                  | Placebo   | 16       | 415   | 107    | 110 | 1520   |                    |            |                 |
| IL-8 (pg/mL)                     | AZD8309   | 15       | 458   | 91     | 69  | 1422   | 0.82               | 0.67, 1.00 | 0.058           |
|                                  | Placebo   | 15       | 559   | 78     | 129 | 1644   |                    |            |                 |
| GRO- $\alpha$ (pg/mL)            | AZD8309   | 16       | 1214  | 78     | 409 | 6020   | 1.09               | 0.95, 1.26 | 0.20            |
|                                  | Placebo   | 16       | 1110  | 65     | 364 | 3220   |                    |            |                 |

\*The results are expressed as the ratio of those after treatment with AZD8309 to those after placebo treatment.

CV, coefficient of variation; Est., estimate; Gmean, geometric mean; NEA, neutrophil elastase activity; LTB4, leukotriene B4; GRO, growth related oncogene- $\alpha$ ; CL, confidence limits.

Table 2. Statistical comparison of nasal lavage data 24 h after lipopolysaccharide challenge

| Variable                          | Treatment | n  | Gmean | CV (%) | Min  | Max  | Analysis of ratio* |            |         |
|-----------------------------------|-----------|----|-------|--------|------|------|--------------------|------------|---------|
|                                   |           |    |       |        |      |      | Est.               | 95% CL     | P-value |
| Leucocytes (10 <sup>4</sup> /mL)  | AZD8309   | 16 | 2.2   | 82     | 0.7  | 7.0  | 0.45               | 0.24, 0.86 | 0.018   |
|                                   | Placebo   | 16 | 4.8   | 75     | 2.0  | 19   |                    |            |         |
| Neutrophils (10 <sup>4</sup> /mL) | AZD8309   | 11 | 2.5   | 88     | 0.69 | 6.3  | 0.57               | 0.24, 1.4  | 0.19    |
|                                   | Placebo   | 11 | 4.4   | 69     | 2.0  | 15   |                    |            |         |
| NEA (10 <sup>3</sup> fluor units) | AZD8309   | 16 | 40    | 56     | 30   | 133  | 0.76               | 0.63, 0.92 | 0.009   |
|                                   | Placebo   | 16 | 52    | 67     | 30   | 197  |                    |            |         |
| LTB4 (pg/mL)                      | AZD8309   | 16 | 111   | 91     | 49   | 527  | 0.70               | 0.50, 0.99 | 0.045   |
|                                   | Placebo   | 16 | 158   | 113    | 50   | 695  |                    |            |         |
| IL-8 (pg/mL)                      | AZD8309   | 16 | 354   | 107    | 75   | 1216 | 0.86               | 0.58, 1.3  | 0.42    |
|                                   | Placebo   | 16 | 411   | 83     | 137  | 1605 |                    |            |         |
| GRO- $\alpha$ (pg/mL)             | AZD8309   | 16 | 891   | 80     | 227  | 2801 | 0.98               | 0.73, 1.3  | 0.89    |
|                                   | Placebo   | 16 | 909   | 72     | 365  | 4682 |                    |            |         |

\*The results are expressed as the ratio of those after treatment with AZD8309 to those after placebo treatment.

CV, coefficient of variation; Est., estimate; Gmean, geometric mean; NEA, neutrophil elastase activity; LTB4, leukotriene B4; GRO, growth related oncogene- $\alpha$ ; CL, confidence limits.

LTB4 and IL-8 [20]. In the present model, we investigated similar parameters to determine whether these measurements of reduced inflammation were replicated upon nasal challenge with LPS after treatment with AZD8309.

Previous studies of bronchial instillation of LPS or inhaled LPS have described a marked neutrophilic inflammation in this model, along with a local secretion of IL-1, IL-6 and TNF- $\alpha$  [21–25], but also systemic effects [26]. In addition, a study by Dosman et al. describes that inhalation of swine dust endotoxin induces both a nasal inflammatory response with the release of IL-8 and an increase of nasal lavage cells, and a systemic response with an increase in blood lymphocytes and in serum levels of IL-6 [27]. In the model where LPS is only applied nasally, a similar cytokine production has been described [28].

We found that the nasal model (LPS installation and nasal lavage) induced only minimal local irritation and no systemic inflammatory signs either clinically or laboratory. The nasal LPS instillation induced an increase in neutrophil count in nasal lavage at 6 h that was still measurable after 24 h. As the cell suspension in lavage consisted of a virtually pure neutrophil population, the quantification of neutrophil egress could be determined directly on the fresh specimen by cell counting in a Bürker chamber and quantified as total leucocyte count. This technique is easier to perform than the differential count on slide smears and shows a possible advantage over the inhaled LPS model. The induced sputum and bronchoalveolar lavage contains a mixed cell population [29, 30], and differential counts are necessary to evaluate to determine the neutrophil count. Another advantage with the nasal challenge model compared with the inhaled LPS model is that

the nasal lavage obtained by the current lavage technique developed in our laboratory resulted in a lavage fluid with very little mucus contamination. Because the lavage fluid contained only small volumes of mucus, the cells could be counted directly without the need for protease treatment of the cell suspension, a procedure that is often necessary in the evaluation of induced sputum [31].

An interaction between the upper and the lower airways is well established, and a familiar feature in allergic rhinitis and asthma [1]. There are many similarities between the nasal and the bronchial mucosa, both morphologically [2] and in the responses to inflammatory stimuli. Previous studies have shown that the nasal and bronchial epithelial cells respond with a similar cytokine pattern in response to pro-inflammatory stimuli [3]. This supports the notion that the responses of the nasal mucosa in the nasal LPS challenge properly reflect those of the bronchial mucosa. Six hours after nasal LPS challenge, we observed a reduction of the amounts of leucocytes and neutrophils and reduced levels of LTB4 after treatment with AZD8309, along with a tendency for decreased levels of neutrophil elastase activity and IL-8. After 24 h, the decrease in leucocyte numbers and levels of LTB4 persisted, and the neutrophil elastase activity was lowered. In spite of the absence of systemic reactions, the cell and mediator results from the nasal challenge are similar to that achieved both after LPS bronchial challenge and induction of sputum with hypertonic saline [20].

Compared with the nasal LPS challenge and nasal lavage technique, the bronchial LPS challenge induced more side effects and is technically more demanding [23, 31, 32]. Furthermore, the processing necessary

for cell count includes a protease treatment of the sample that also may influence the assays for mediator determination [33, 34]. Our study did not show an increased inflammatory response after repeated nasal lavages. This is in contrast to the induced sputum procedure that is not readily repeated because the procedure itself induces an inflammatory reaction [4, 34]. While we observed a neutrophilic inflammation in the nose upon LPS challenge, it should be noted that the type of inflammatory response occurring in the nose is dependent on the applied stimuli. Other stimuli have been shown to induce an influx of monocytes, lymphocytes and eosinophils to the nose [35–37]. The present model of nasal LPS challenge is therefore most suitable to investigate neutrophilic inflammation, but is limited in regard to study of other cell types.

The technique applied by us showed a reduced leucocyte count after treatment with AZD8309, along with a reduction in LTB<sub>4</sub> activity, and trends for reductions in

neutrophil elastase activity and IL-8. This demonstrates that AZD8309 inhibits LPS-induced nasal inflammation, in a manner which mimics the local response seen in the previous study of the lower airway, responses to inhaled LPS. The nasal model appears to be a well tolerated and convenient way to evaluate the effects of novel anti-inflammatory drugs affecting neutrophilic migration and activity.

### Acknowledgements

The authors would like to thank Ann Reutherborg, Tomas Ottoson, Carita Lindqvist and Eva Lindqvist for skilful technical assistance during the course of this study.

*Conflict of interest:* A. K. E., U. W. and L. O. C. have no conflicts of interest. L. J. is a former and R. V. is a present employee of AstraZeneca, the owner of the drug AZD8309.

### References

- Bousquet J, Khaltaev N, Cruz AA *et al*. Allergic rhinitis and its impact on asthma (aria) 2008 update (in collaboration with the world health organization, ga(2)len and allergen). *Allergy* 2008; **63**(Suppl. 86):8–160.
- Devalia JL, Sapsford RJ, Wells CW, Richman P, Davies RJ. Culture and comparison of human bronchial and nasal epithelial cells in vitro. *Respir Med* 1990; **84**:303–12.
- McDougall CM, Blaylock MG, Douglas JG, Brooker RJ, Helms PJ, Walsh GM. Nasal epithelial cells as surrogates for bronchial epithelial cells in airway inflammation studies. *Am J Respir Cell Mol Biol* 2008; **39**:560–8.
- Kharitonov SA, Sjobring U. Lipopolysaccharide challenge of humans as a model for chronic obstructive lung disease exacerbations. *Contrib Microbiol* 2007; **14**:83–100.
- Singh J, Schwartz DA. Endotoxin and the lung: insight into the host-environment interaction. *J Allergy Clin Immunol* 2005; **115**:330–3.
- Togbe D, Schnyder-Candrian S, Schnyder B *et al*. Tlr4 gene dosage contributes to endotoxin-induced acute respiratory inflammation. *J Leukoc Biol* 2006; **80**:451–7.
- Thorn J. The inflammatory response in humans after inhalation of bacterial endotoxin: a review. *Inflamm Res* 2001; **50**:254–61.
- Haddad EB, Birrell M, McCluskie K *et al*. Role of p38 map kinase in lps-induced airway inflammation in the rat. *Br J Pharmacol* 2001; **132**:1715–24.
- Thorn J, Rylander R. Inflammatory response after inhalation of bacterial endotoxin assessed by the induced sputum technique. *Thorax* 1998; **53**:1047–52.
- Ekman AK, Fransson M, Rydberg C, Adner M, Cardell LO. Nasal challenge with lps stimulates the release of macrophage inflammatory protein 1alpha. *Int Arch Allergy Immunol* 2009; **149**:154–60.
- Maris NA, de Vos AF, Dessing MC, *et al*. Antiinflammatory effects of salmeterol after inhalation of lipopolysaccharide by healthy volunteers. *Am J Respir Crit Care Med* 2005; **172**:878–84.
- Haddad e-B, McCluskie K, Birrell MA *et al*. Differential effects of ebselen on neutrophil recruitment, chemokine, and inflammatory mediator expression in a rat model of lipopolysaccharide-induced pulmonary inflammation. *J Immunol* 2002; **169**:974–82.
- Shyamsundar M, McKeown ST, O'Kane CM *et al*. Simvastatin decreases lipopolysaccharide-induced pulmonary inflammation in healthy volunteers. *Am J Respir Crit Care Med* 2009; **179**:1107–14.
- Rocksén D, Lilliehook B, Larsson R, Johansson T, Bucht A. Differential anti-inflammatory and anti-oxidative effects of dexamethasone and n-acetylcysteine in endotoxin-induced lung inflammation. *Clin Exp Immunol* 2000; **122**:249–56.
- Michel O, Dentener M, Cataldo D *et al*. Evaluation of oral corticosteroids and phosphodiesterase-4 inhibitor on the acute inflammation induced by inhaled lipopolysaccharide in human. *Pulm Pharmacol Ther* 2007; **20**:676–83.
- Belvisi MG. Regulation of inflammatory cell function by corticosteroids. *Proc Am Thorac Soc* 2004; **1**:207–14.
- Fransson M, Adner M, Uddman R, Cardell LO. Lipopolysaccharide-induced down-regulation of uteroglobin in the human nose. *Acta Otolaryngol* 2007; **127**:285–91.
- Murphy PM. Neutrophil receptors for interleukin-8 and related cxc chemokines. *Semin Hematol* 1997; **34**:311–8.
- White JR, Lee JM, Young PR *et al*. Identification of a potent, selective non-peptide cxcr2 antagonist that inhibits interleukin-8-induced neutrophil migration. *J Biol Chem* 1998; **273**:10095–8.
- O'Connor BLBP, Nicholson G, Graham C *et al*. Inhibition of lps-induced neutrophilic inflammation in healthy volunteers. Abstract 1294. Stockholm, Sweden: ERS, 2007.
- Sandstrom T, Bjermer L, Rylander R. Lipopolysaccharide (lps) inhalation in healthy subjects causes bronchoalveolar neutrophilia, lymphocytosis, and

- fibronectin increase. *Am J Ind Med* 1994; 25:103–4.
- 22 Blaski CA, Clapp WD, Thorne PS *et al.* The role of atopy in grain dust-induced airway disease. *Am J Respir Crit Care Med* 1996; 154:334–40.
  - 23 Nightingale JA, Rogers DF, Hart LA, Kharitonov SA, Chung KF, Barnes PJ. Effect of inhaled endotoxin on induced sputum in normal, atopic, and atopic asthmatic subjects. *Thorax* 1998; 53:563–71.
  - 24 O'Grady NP, Preas HL, Pugin J *et al.* Local inflammatory responses following bronchial endotoxin instillation in humans. *Am J Respir Crit Care Med* 2001; 163:1591–8.
  - 25 Michel O. Role of lipopolysaccharide (lps) in asthma and other pulmonary conditions. *J Endotoxin Res* 2003; 9:293–300.
  - 26 Michel O, Dentener M, Corazza F, Burman W, Rylander R. Healthy subjects express differences in clinical responses to inhaled lipopolysaccharide that are related with inflammation and with atopy. *J Allergy Clin Immunol* 2001; 107:797–804.
  - 27 Dosman JA, Fukushima Y, Senthilselvan A *et al.* Respiratory response to endotoxin and dust predicts evidence of inflammatory response in volunteers in a swine barn. *Am J Ind Med* 2006; 49:761–6.
  - 28 Sigsgaard T, Bonefeld-Jorgensen EC, Kjaergaard SK, Mamas S, Pedersen OF. Cytokine release from the nasal mucosa and whole blood after experimental exposures to organic dusts. *Eur Respir J* 2000; 16:140–5.
  - 29 Pin I, Gibson PG, Kolendowicz R *et al.* Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992; 47:25–9.
  - 30 Schaumann F, Muller M, Braun A *et al.* Endotoxin augments myeloid dendritic cell influx into the airways in patients with allergic asthma. *Am J Respir Crit Care Med* 2008; 177:1307–13.
  - 31 Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. *Thorax* 1997; 52:498–501.
  - 32 Michel O, Nagy AM, Schroeven M *et al.* Dose-response relationship to inhaled endotoxin in normal subjects. *Am J Respir Crit Care Med* 1997; 156:1157–64.
  - 33 Magnussen H, Holz O. Monitoring airway inflammation in asthma by induced sputum. *Eur Respir J* 1999; 13:5–7.
  - 34 Antczak A, Kharitonov SA, Montuschi P, Gorski P, Barnes PJ. Inflammatory response to sputum induction measured by exhaled markers. *Respiration* 2005; 72:594–9.
  - 35 Ying S, Durham SR, Jacobson MR *et al.* T lymphocytes and mast cells express messenger rna for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. *Immunology* 1994; 82:200–6.
  - 36 Kuna P, Alam R, Ruta U, Gorski P. Rantes induces nasal mucosal inflammation rich in eosinophils, basophils, and lymphocytes in vivo. *Am J Respir Crit Care Med* 1998; 157:873–9.
  - 37 Lonnkvist K, Moshfegh A, Pedroletti C, Hedlin G, Hallden G, Lundahl J. Increased eosinophil transmigration after nasal allergen challenge in children with allergic asthma and rhinitis. *Allergy* 2002; 57:1200–4.