Editor's Choice

The Editor takes a closer look at some of this month's articles

Aspirin induced asthma: lipoxins versus leukotrienes. Aspirin intolerant asthma continues to be a fascinating, but poorly understood sub-phenotype of asthma. It is well established that there is an excess of sulphidopeptide leukotrienes in this condition with particular increased amounts of urinary leukotriene E_{4} (LTE_{4}). However the potential involvement of an anti-inflammatory class of eicosanoids, the lipoxins, has not been examined in any detail. Lipoxins are eicosanoids (mediators derived from arachidonic acid), that include lipoxin A_{4} (LXA_{4}), B_{4} (LXB_{4}) and the epi-lipoxins formed by non-enzymatic peroxidation of lipoxin. Lipoxins inhibit leucocyte function and are thought to play a role in the resolution of inflammation [1]. In this issue Yamaguchi and colleagues (pp. 1711–1718) have investigated the urinary concentrations of lipoxin A_{4}, 15 epi-LXA_{4} and LTE_{4} in patients with aspirin intolerant (AIA) and tolerant asthma (ATA) as well as normal controls. Consistent with other studies they found LTE_{4} concentrations to be higher in the AIA subjects. Overall in asthma they found urinary concentrations of 15 epi-LXA_{4} to be higher than LXA_{4}. They found reduced amounts of 15 epi-LXA_{4} in AIA, which was not related to disease severity, suggesting that AIA is associated with an imbalance between pro and anti-inflammatory eicosanoids. This paper adds to the growing body of evidence suggesting that lipoxins may have a role as therapeutic agents in asthma and related diseases.

Diet and allergic disease: more on the fatty acid story. There continues to be considerable interest in the possible relationship between the intake of fatty acids and allergic disease [2]. Increased consumption of n-6 polysaturated fatty acids (PUVA) through the production of arachidonic acid may have pro-inflammatory consequences, as oppose to consumption of n-3 PUVA which generate alternative long chain fatty acids such as eicosapentanoic acid, which inhibit AA production and are linked to the production of the anti-inflammatory resolvins. However the literature on this subject remains contradictory. Standl et al. (pp. 1757–1766) asked in two birth cohort studies whether this could be due to variations in the metabolism of fatty acids which is under tight genetic control, including by the products of the fatty acid desaturase (FADS) 1 and 2 genes. They found that margarine intake was significantly linked to asthma in children with the homozygous major allele with a borderline relationship between the N-3/N-6 ratio and the incidence of hayfever in the children with the homozygous major allele. More evidence that when considering environmental influences on allergic disease, the genetic background has to be taken into account.

New treatments for asthma: a novel approach to Th2 blockade. The hypothesis that asthma is primarily due to recruitment of inflammatory cells, particularly eosinophils, into the lung as a result of the release of cytokines related to activated Th2 lymphocytes, resulted in a considerable effort by the pharmaceutical industry to block these pathways. The fruits of this endeavour are just now appearing as clinical trials in the literature. Imaoka et al. (pp. 1740–1746) report further data on a novel inhaled preparation, TPI ASM8, that contains two antisense oligonucleotides targeted at blocking eosinophil and possibly basophil recruitment into the lung. One oligo is directed against the common beta chain of the IL-3/IL-5/GM-CSF cytokines and the other against the eosinophil chemoattractant CCR3. It therefore has in theory a much broader mechanism of action than single agents such as mepolizumab which just blocks IL-5. The authors previously demonstrated that this compound had efficacy in an allergen challenge model [3], and here they show that it also blocks the recruitment of eosinophil precursors which are thought to be a significant part of the eosinophil inflammatory process in asthma. This and other drugs like it offer real hope for people with eosinophilic asthma in the relatively near future.

References
1 Boyce JA. Eicosanoids in asthma, allergic inflammation, and host defense. Curr Mol Med 2008; 8:335-49.

Caption to cover illustration: Space filling CPK and ball-and-stick three-dimensional molecular models illustrating the encapsulation of rocuronium by sugammedex to form a sugammedex-rocuronium inclusion complex (see fig 5, B. A. Baldo et al, pp. 1663–1678)
TPI ASM8 reduces eosinophil progenitors in sputum after allergen challenge

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Summary
Background TPI ASM8 contains two modified phosphorothioate antisense oligonucleotides (AON), one targeting the common beta chain (βc) of the IL-3/IL-5/GM-CSF receptors and the other targeting the chemokine receptor CCR3. Inhalation of TPI ASM8 significantly improves lung function and sputum eosinophilia after allergen inhalation challenge in asthmatics.

Objective This study assessed whether TPI ASM8 reduces airway levels of haemopoietic progenitor cells.

Methods This open-label study was conducted in 14 stable, allergic mild asthmatic subjects with early- and late-phase allergen-induced bronchoconstriction. Subjects underwent allergen challenges after 4-day treatment with placebo, 4 mg b.i.d. and 8 mg o.d. of TPI ASM8. Sputum was induced before, 7 and 24 h after allergen challenges for progenitor measurements. Treatments were separated by 2–3 weeks.

Results TPI ASM8 reduced allergen-induced sputum eosinophils, and the early and late asthmatic responses (P < 0.05). TPI ASM8 also reduced the number of CD34+CCR3+ cells (P = 0.004) and CD34+IL-5Rα+ cells (P = 0.016), and the proportion of CD34+ cells expressing IL-5Rα (P = 0.036).

Conclusions and Clinical Relevance TPI ASM8 was safe and well tolerated. The results of this study demonstrate blocking of CCR3 and βc expression by TPI ASM8 significantly inhibits the accumulation of eosinophils and eosinophil progenitors in the airways after allergen challenge. Inhibition of airway progenitor cell accumulation presents a novel therapeutic target.

Keywords airway inflammation, allergen inhalation, allergic asthma, antisense oligonucleotides, eosinophils, progenitor mobilization

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Introduction
Haemopoietic myeloid progenitors contribute to the ongoing recruitment of pro-inflammatory cells, such as eosinophils and basophils, to target tissue sites in allergic diseases. It is evident that the development of allergic inflammation is associated with the ability of the bone marrow and affected tissues to support the mobilization, proliferation and in situ differentiation of haemopoietic progenitors.

In humans, bone marrow-derived CD34+CD45+ haemopoietic progenitor cells (HPC) are higher in the circulation of subjects with allergic asthma compared with controls [1] and have been detected in different airway compartments of allergic individuals including mucosa of the upper and lower airways and airway secretions [2–7]. The number of HPC in sputum samples from asthmatic donors is further enhanced following allergic stimulation of the airways [4]. In mouse models of allergic asthma, lung-extracted HPC differentiated into eosinophils thus demonstrating another mechanism for rapidly increasing the local level of mature eosinophils in tissue during an inflammatory response [8].

The chemokine eotaxin and the cytokine IL-5 are critical for mobilization and differentiation, respectively, of eosinophils [9]. Thus, expression of receptors for these mediators is another critical component of tissue infiltration. A thorough examination of eosinophils from bone marrow samples shows higher expression of CCR3 in subjects with asthma vs. control subjects, suggesting there is an increased pool of CCR3+ mature and immature eosinophils in subjects with asthma which can be rapidly mobilized following appropriate stimulation [10]. Fluctuations in CCR3 expression on human bone marrow CD34+ cells may also facilitate chemokine-mediated progenitor cell mobilization and differentiation to the peripheral circulation, as evidenced by increased levels of CCR3 and IL-5Rα-subunit expression on...
bone marrow CD34+ cells from donors following the development of a late asthmatic response to inhaled allergen [11–13]. Local IL-5-dependent differentiation of progenitor cells has been observed in nasal mucosa; \textit{ex vivo} culture with IL-5 or allergen showed a reduction in CD34+/IL-5Rα mRNA+ cell numbers and a concurrent increase the number of MBP immunoreactive cells, demonstrating that a subset of eosinophils (and possibly MBP-positive basophils) can differentiate within the explanted tissue [3]. Similar observations have been reported in bronchial biopsies taken from asthmatics following IL-5 inhalation [14].

While it is evident that in asthma the lung tissue can provide a microenvironment suitable for extramedullary eosinophilopoiesis and basophilopoiesis, the effect of targeting critical cytokine and chemokine pathways to control tissue infiltration of progenitors in asthmatic subjects has not been investigated to date. TPI ASM8 is a drug product containing two modified phosphorothioate antisense oligonucleotides (AON): TOP004 directed against human βc of IL-3, IL-5 and GM-CSF receptors, and TOP005 directed against human CCR3 [15]. These AONs down-regulate the transcription of CCR3 and βc, and previously have been shown to inhibit allergen-induced eosinophilia and airway dysfunction in sensitized rats [16–18] and human asthmatics [19]. Here, we hypothesized TPI ASM8 could inhibit the migration of eosinophil progenitors into the airways. This was assessed by comparing the level of HPC in the migration of eosinophil progenitors into the airways.

\textbf{Methods}

\textbf{Subjects}

Fourteen subjects entered the baseline period. Subjects were non-smoking men and women (eight males/six females), aged 19–58 years old, with mild atopic stable asthma. Subjects had a forced expiratory volume in 1 s (FEV$_1$) was ≥70% of predicted and a measureable methacholine PC$_{20}$ (the provocative concentration of methacholine causing a 20% fall in FEV$_1$). Subjects had no other lung disease, no lower respiratory tract infection or worsening of asthma within 6 weeks, and avoided exposure to sensitizing allergens apart from house dust mite. Subjects were steroid naive, infrequently used inhaled β$_2$-agonist for treatment of asthma, and refrained from β$_2$-agonist and caffeinated beverages before laboratory visits. Of those that met the inclusion/exclusion criteria, 12 subjects completed all of the study treatment periods. Subject demographics are shown in Table 1.

\textbf{Study design}

This study was designed as a single center, placebo-controlled, open-label study comparing two doses of inhaled TPI ASM8 (Topigen Pharmaceuticals Inc., Montreal, QC, Canada) to placebo on efficacy outcomes and safety for the treatment of allergen-induced asthma. The study was approved by the McMaster Faculty of Health Sciences/Hamilton Health Sciences Research Ethics Board and signed informed consent was obtained from subjects. The study was divided into two periods. During the baseline period subjects underwent allergen challenges to document early- and late-phase bronchoconstriction. During this baseline period, subjects practised dosing by inhaling saline nebulized using the 1-neb™ (Philips Respironics, Pittsburgh, PA, USA) for 4 days. Baseline allergen challenge was conducted on the morning of the fourth day of saline treatment, and thus served as a placebo control for the study (Fig. 1).

Subjects demonstrating early-phase (> 20% fall in FEV$_1$ 0–2 h post-allergen) and late-phase (> 15% fall in FEV$_1$ 3–7 h post-allergen) responses, and with sputum eosinophil levels >6% at 7 h or 24 h post-allergen, met the criteria to receive active treatment with TPI ASM8. These subjects entered a 2-week washout period after

**Table 1. Subject demographics collected during the baseline screening period**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Gender</th>
<th>Predicted FEV$_1$(%)</th>
<th>Methacholine PC$_{20}$(mg/mL)</th>
<th>EAR (% change)</th>
<th>LAR (% change)</th>
<th>Allergen extract</th>
<th>Cumulative dose (BAU)</th>
<th>Sputum eosinophils (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>F</td>
<td>91.2</td>
<td>0.50</td>
<td>−26.0</td>
<td>−16.8</td>
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<td>6.4</td>
</tr>
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<td>M</td>
<td>98.0</td>
<td>5.79</td>
<td>−28.6</td>
<td>−21.4</td>
<td>HDMDP</td>
<td>228.5</td>
<td>1.3</td>
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<tr>
<td>3</td>
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<td>F</td>
<td>109.4</td>
<td>3.13</td>
<td>−36.8</td>
<td>−25.3</td>
<td>Cat</td>
<td>304.7</td>
<td>3.6</td>
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<tr>
<td>4</td>
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<td>M</td>
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<td>4.78</td>
<td>−38.9</td>
<td>−28.9</td>
<td>HDMDF</td>
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<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>M</td>
<td>87.3</td>
<td>1.36</td>
<td>−31.1</td>
<td>−23.0</td>
<td>Alternaria</td>
<td>487.5</td>
<td>3.0</td>
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<tr>
<td>6</td>
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<td>F</td>
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<td>2.83</td>
<td>−39.7</td>
<td>−15.9</td>
<td>HDMDF</td>
<td>106.6</td>
<td>0.9</td>
</tr>
<tr>
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<td>M</td>
<td>97.2</td>
<td>2.38</td>
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<td>−15.0</td>
<td>Grass</td>
<td>243.8</td>
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<td>M</td>
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<td>3.56</td>
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<td>HDMDF</td>
<td>213.3</td>
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<td>F</td>
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<td>5.74</td>
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<td>F</td>
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<td>M</td>
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<td>−19.0</td>
<td>Grass</td>
<td>1137.5</td>
<td>0.2</td>
</tr>
<tr>
<td>12</td>
<td>58</td>
<td>M</td>
<td>97.2</td>
<td>19.6</td>
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<td>−20.5</td>
<td>Cat</td>
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</tr>
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</table>

FEV$_1$, forced expiratory volume in 1 s; PC$_{20}$, provocative concentration causing a 20% fall in FEV$_1$; EAR, early asthmatic response; LAR, late asthmatic response; BAU, bioequivalent allergy unit.
which asthma stability criteria (FEV\textsubscript{1} within 10\% of baseline and methacholine PC\textsubscript{20} within one doubling dose of baseline) were required in order to proceed with the first dose of medication. Subjects inhaled 4 mg TPI ASM8 b.i.d. for four consecutive days and allergen challenge was conducted on Day 4. After a 2-week washout subjects inhaled 8 mg TPI ASM8 o.d. for four consecutive mornings and allergen challenge was conducted on Day 4. Morning doses on Days 1, 3 and 4 were administered in the laboratory. On Day 3, subjects underwent sputum induction 45 min after the morning dosing. On Day 4, subjects underwent allergen challenge 45 min after the morning dosing, and spirometry was measured at regular intervals until 7 h post-challenge when sputum was induced. Subjects returned the following morning on Day 5 for sputum induction (Fig. 1). The current study evaluated sputum eosinophil progenitors levels after allergen challenge at each dose of TPI ASM8 compared with placebo. Sputum differential cell counts and early- and late-phase bronchoconstriction were also compared with placebo.

**Laboratory procedures**

**Study medication.** TPI ASM8 contains two phosphorothioate AONs, TOP 004 and TOP 005 at a 1 : 1 ratio by weight. The nucleotide sequence of each of the two AONs in TPI ASM8 is as follows TOP 004: 5’-GGGTCTGCXGCGGGXTGGT and TOP 005: 5’-GGGTCTGCXGCGGGXTGGT where X is 2-amino-2’deoxyadenosine. TPI ASM8 solution was prepared in 0.5 mL normal saline. Inhalation of 0.5 mL took approximately 5 min. Placebo was 0.5 mL normal saline.

**Methacholine inhalation.** Methacholine inhalation challenge was performed as described by Cockcroft [20], using tidal breathing, from a Wright nebulizer. The test was terminated when a fall in FEV\textsubscript{1} of at least 20\% of the baseline value occurred, and the methacholine PC\textsubscript{20} was calculated.

**Allergen inhalation challenge.** Allergen challenge was performed as described by O’Byrne and colleagues [21]. The concentration of allergen extract for inhalation was determined from a formula described by Cockcroft and colleagues [22]. The baseline allergen challenge administered doubling concentrations of allergen until a $\geq 20\%$ fall in FEV\textsubscript{1} at 10 min post-inhalation was reached. The FEV\textsubscript{1} was then measured at regular intervals until 7 h after allergen inhalation. The maximum percent fall in FEV\textsubscript{1} was recorded for the early (0–2 h post-allergen) and late (3–7 h post-allergen) phase responses. The same dose of allergen was used for all three allergen challenges.

**Sputum induction.** Sputum was induced and processed using the method described by Pizzichini and colleagues [23]. Cytospins were prepared on glass slides and stained with Diff Quik (American Scientific Products, McGaw Park, IL, USA) for differential counts, which were enumerated using an observer blinded to the treatment period. The remaining sputum cell suspension was used for measurements of HPC.

**Flow cytometry.** Sputum cells were stained with antibodies to surface markers [CD45-fluorescein isothiocyanate, CD34-allophycocyanin and CDw125 (IL-5R\textsubscript{1})-phycoerythrin (PE): BD Biosciences, San Jose, CA, USA; CCR3-PE: MBL, Woburn, MA, USA]. The level of surface staining was determined from a formula described by Cockcroft and colleagues [22]. The baseline allergen challenge administered doubling concentrations of allergen until a $\geq 20\%$ fall in FEV\textsubscript{1} at 10 min post-inhalation was reached. The FEV\textsubscript{1} was then measured at regular intervals until 7 h after allergen inhalation. The maximum percent fall in FEV\textsubscript{1} was recorded for the early (0–2 h post-allergen) and late (3–7 h post-allergen) phase responses. The same dose of allergen was used for all three allergen challenges.

**Statistical analysis**

Results are expressed as mean±standard error of the mean (SEM), with the exception of sputum HPC/mL, which are expressed as geometric mean±GSEM. Sputum HPC/mL values were log\textsubscript{10} transformed to fit a normal distribution before analysis. The early-phase (EAR) and late-phase asthmatic responses (LAR) and the number of sputum eosinophils were compared with the corresponding time-
point during placebo treatment using a Wilcoxon signed-rank test (two-sided). Sputum HPC were analysed using a two-way analysis of variance (Statistica v7.0), with main factors treatment, time, and treatment×time. Post hoc tests for pre-planned comparisons were carried out.

Results

Airway responses

The maximum percent fall in FEV₁ during the EAR with placebo treatment was 32.5±1.8% and during the LAR was 22.8±1.8%. Treatment with 4 mg b.i.d. and 8 mg o.d., significantly attenuated the LAR to 12.8±1.8% (P = 0.0008) and 10.6±2.0% (P = 0.00007), respectively, compared with placebo. Although there was no significant inhibition of the EAR with 4 mg b.i.d., treatment with 8 mg o.d. of TPI ASM8 significantly attenuated the EAR to 21.5±3.6% compared with placebo (P = 0.006).

Sputum eosinophils

Sputum eosinophils increased significantly from 0.15±0.07×10⁶/mL pre-allergen to 1.10±0.17×10⁶/mL and 1.24±0.28×10⁶/mL at 7 and 24 h post-allergen, respectively (P<0.05) with placebo. Treatment with 8 mg o.d. of TPI ASM8 significantly inhibited allergen-induced sputum eosinophils to 0.46±0.13×10⁶/mL at 7 h and 0.35±0.04×10⁶/mL 24 h after allergen inhalation challenge (P = 0.005 and P = 0.04, respectively). The numeric decrease in sputum eosinophils to 0.87±0.20×10⁶/mL at 7 h and 0.53±0.14×10⁶/mL at 24 h after allergen challenge during 4 mg b.i.d. treatment did not reach statistical significance (P>0.05).

Haemopoietic progenitor cells in sputum

HPC were enumerated in sputum samples using logical sequential multi-gating flow cytometric analyses (Fig. 2). Treatment with 4 mg TPI ASM8 b.i.d. had no effect on the numbers of total CD34⁺ HPC within the sputum (Fig. 3). Although treatment with 8 mg TPI ASM8 o.d. showed a trend for reduction of the absolute number of sputum CD34⁺ cells at each time-point, this was not statistically significant.

Analyses of subpopulations of the HPC showed that there was a significant reduction in the absolute number of eosinophil progenitors (CD34⁺IL-5Rα⁺ cells) with 8 mg o.d. compared with placebo (P = 0.008, Fig. 4a). Pre-planned comparisons of CD34⁺IL-5Rα⁺ cells at baseline and 24 h post-allergen were significantly lower with 8 mg o.d. treatment compared with placebo; placebo baseline 302 cells/mL (GSEM 102–155) vs. 8 mg o.d. baseline 159 cells/mL (GSEM 38–50) (P = 0.035), placebo 24 h post-allergen 437 cells/mL (GSEM 148–224) vs. 8 mg o.d. 24 h post-allergen 138 cells/mL (GSEM 33–44) (P = 0.0499). There was no effect of 4 mg b.i.d. on the absolute number of CD34⁺IL-5Rα⁺ cells (P = 0.91) (Fig. 4a).

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There was a significant effect of 4 mg b.i.d. on the proportion of sputum CD34\(^+\) cells expressing IL-5R\(\alpha\) \((P = 0.015)\) and a trend for an effect of 8 mg o.d. \((P = 0.065, \text{Fig. 4b})\). Post hoc analyses of pre-planned comparisons between 4 mg b.i.d. and placebo showed TPI ASM8 reduced the percentage of sputum CD34\(^+\) cells expressing IL-5R\(\alpha\) at 7 h \((P = 0.014)\) and a trend for reduction at baseline \((P = 0.07, \text{Fig. 4b})\).

Up-regulation of CCR3 on CD34\(^+\) is associated with increased migrational responsiveness of progenitor cells [11]. In this study, there was a significant reduction in the absolute number of sputum-derived CD34\(^+\)CCR3\(^+\) cells with 8 mg o.d. compared with placebo \((P = 0.002, \text{Fig. 4c})\). Pre-planned comparisons of CD34\(^+\)CCR3\(^+\) cells at baseline and 24 h post-allergen were significantly lower with 8 mg o.d. treatment compared with placebo; placebo baseline 251 cells/mL (GSEM 77–112) vs. 8 mg o.d. baseline 71 cells/mL (GSEM 36–74) \((P = 0.007)\), placebo 24 h post-allergen 132 cells/mL (GSEM 67–137) vs. 8 mg o.d. 24 h post-allergen 25 cells/mL (GSEM 13–29) \((P = 0.013)\).

Fig. 3. The absolute number of CD34\(^+\) cells were enumerated in sputum samples at baseline, 7 and 24 h post-allergen inhalation challenge following treatment with placebo, 4 mg b.i.d. and 8 mg o.d. of TPI ASM8. Data are presented as geometric mean\pm GSEM. Treatment with TPI ASM8 had no effect on total haemopoietic cell (HPC) numbers in the sputum.

Fig. 4. (a) The absolute number of CD34\(^+\)IL-5R\(\alpha\)\(^+\) cells; (b) the percentage of CD34\(^+\) cells expressing IL-5R\(\alpha\)\(^+\); (c) the absolute number of CD34\(^+\)CCR3\(^+\) cells; and (d) the percentage of CD34\(^+\) cells expressing CCR3 in sputum samples at baseline, 7 and 24 h post-allergen inhalation challenge following treatment with placebo, 4 mg b.i.d. and 8 mg o.d. of TPI ASM8. Percentages are presented as mean\pm SEM and cells/ml are presented as geometric mean\pm GSEM. *\(P<0.05\) for pre-planned comparisons between placebo and TPI ASM8 treatment.

Safety

There were no serious adverse events (AEs) in this study. Seventeen AEs were reported; three AEs occurred during the placebo/screening period, one during the 4 mg b.i.d.
period, and four during the 8 mg o.d. period. The most frequently reported AE was headache, and only one AE, shortness of breath during the 8 mg o.d. dose was deemed to be possibly drug.

Discussion

In this study, we report the effect of RNA-targeted topical therapy on mature eosinophils and HPC in sputum samples from dual responder asthmatics. TPI ASM8 contains two AONs (TOP004 and TOP005), which down-regulate mRNA transcripts for βc and CCR3 [15–17] leading to a reduction of these receptors on the cell surface. A previous study has reported that treatment with TPI ASM8 attenuated allergen induced airway responses and airway eosinophilia[19]. In the current study, we report that treatment with TPI ASM8 had a significant effect on lowering not only mature eosinophils but also eosinophil/basophil progenitor cell populations in sputum. Analyses of data from 12 asthmatic subjects showed that the drug had no effect on the total number of HPC within the sputum, but there was a significant reduction in CD34⁺ IL-5Rα⁺ cells numbers (P = 0.016) and CD34⁺ CCR3⁺ cells numbers (P = 0.004). In addition, when the data were expressed as a percentage of the total HPC population, we detected a reduction in the proportion of CD34⁺ cells expressing IL-5Rα (P = 0.036) following pre-treatment with by TPI ASM8. There were trends for reduction of the proportion of CD34⁺ cells expressing CCR3 (P = 0.06). The current data show that TPI ASM8 has effects that are specific for progenitors of eosinophil/basophil lineage. The effects observed with TPI ASM8 administered at a dose of 8 mg o.d. but not 4 mg b.i.d. may be due to long-term carryover effects of drug into the next treatment period, even though the washout period between periods was sufficient for complete drug washout from the airways.

Dorman et al. [4] have previously reported increased HPC and eosinophil progenitors (CD34⁺ IL-5Rα⁺) in sputum after allergen inhalation challenge in dual responder asthmatics. Our current findings demonstrate a trend for an increase in the absolute number of CD34⁺ cells and CD34⁺ IL-5Rα⁺ cells following allergen challenge in the placebo arm of the study, but these changes were not significant. This may reflect the increased variability within this population of asthmatics and a larger sample size may have shown a significant allergen-induced effect, although this was not a primary outcome of this study.

Expression of CCR3 and the common βc of IL-5, IL-3 and GM-CSF are critical for eosinophil and basophil progenitor cells to migrate and proliferate in tissue. Studies have previously shown that up-regulation of CCR3 on CD34⁺ cells was detected on bone marrow progenitor cells following allergen challenge and that this was associated with migrational increased responsiveness to eotaxin, in vitro[11, 24]. As expected, treatment with TPI ASM8 reduced the level of CCR3 expression on CD34⁺ cells in the sputum as this was one of the drug targets. However, although the drug also targets βc mRNA, we found a reduction in the number of CD34⁺ cells expressing the α-chain of the IL-5 receptor. The IL-5 receptor is a heterodimeric receptor composed of a specific binding α-chain and the β-chain that is the signaling component shared with IL-3 and GM-CSF [25]. Studies have shown that IL-5 itself can regulate the expression of its own α-chain expression receptor on CD34⁺ cells [26]. Thus down-regulation of the signalling component of the IL-5R (i.e. βc chain) is likely to directly impact the reduction in IL-5Rα-chain expression on CD34⁺ cells as has been shown in this study. We believe the decreased number of eosinophil progenitor cells in the airways is due to the effects of this drug on the cells that have already been recruited to the airways, because there is no detectable level of drug present in the circulation of these subjects post-dosing (data not shown).

Studies with anti-IL-5 have shown no effects on baseline levels of CD34⁺ IL-5Rα⁺ cells in bone marrow and blood and only a partial reduction in CD34⁺ IL-5Rα mRNA⁺ cells in lung biopsies following intravenous infusions of mepolizumab [27]. No studies in humans have looked at the effect of modifying either IL-5- or CCR3-mediated pathways on the lung homing of HPC following allergen challenge. In the current study, we show for the first time that TPI ASM8 can selectively modulate the lung levels of specific HPC phenotype and that this may contribute to the observed marked reduction in lung eosinophilia.

In summary, we conclude that in addition to directly targeting mature granulocytes, TPI ASM8 may regulate allergen-induced lung eosinophilia and basophilia through a reduction in expression of CCR3 and βc-chain on HPC within the airways.

Acknowledgements

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References

3 Cameron L, Christodoulopoulos P, Lavigne F et al. Evidence of local


16 Allakhverdi Z, Allam M, Renzi PM. Inhibition of allergen-induced eosinophilia and airway hyperresponsiveness by antisense oligonucleotides directed against the common beta chain of IL-3, IL-5, GM-CSF receptors in a rat model of allergic asthma. *Am J Respir Crit Care Med* 2002; 165: 1015–21.


