Effect of cilomilast (Ariflo) on TNF-α, IL-8, and GM-CSF release by airway cells of patients with COPD

M Profita, G Chiappara, F Mirabella, R Di Giorgi, L Chimenti, G Costanzo, L Riccobono, V Bellia, J Bousquet, A M Vignola

**Background:** Inflammation in chronic obstructive pulmonary disease (COPD) is characterised by increased neutrophilic infiltration of the airways. Cilomilast, a novel selective phosphodiesterase 4 inhibitor in clinical development for COPD treatment, exerts anti-inflammatory effects. The ability of cilomilast to inhibit the release of neutrophil chemoattractants such as tumour necrosis factor (TNF-α), interleukin (IL)-8, and granulocyte-macrophage colony stimulating factor (GM-CSF) by bronchial epithelial cells and sputum cells isolated from 10 patients with COPD, 14 normal controls, and 10 smokers was investigated.

**Methods:** Bronchial epithelial cells obtained by bronchial brushing and sputum cells isolated from induced sputum samples were cultured for 24 hours in the presence or absence of cilomilast (1 µM). After incubation the supernatants were harvested and the levels of mediators measured by ELISA.

**Results:** TNF-α and IL-8 release by bronchial epithelial cells and sputum cells was higher in patients with COPD than in controls (p<0.0001) and smokers (p<0.0001). GM-CSF was only detectable in sputum cell supernatants and its level was higher in patients with COPD than in controls and smokers (p<0.0001, respectively). Cilomilast significantly reduced TNF-α release by bronchial epithelial cells and sputum cells (p=0.005) and GM-CSF release by sputum cells (p=0.003), whereas IL-8 release was not statistically inhibited. Supernatants of sputum cells and bronchial epithelial cells treated with cilomilast significantly decreased neutrophil chemotaxis (p<0.006 and p<0.008, respectively).

**Conclusions:** Cilomilast inhibits the production of some neutrophil chemoattractants by airway cells. This drug may play a role in the resolution of neutrophilic inflammation associated with COPD and cigarette smoke.

Cilomilast (Ariflo, SB-207499) is an orally active second generation PDE4 inhibitor that may be effective in the treatment of COPD. It was found to reduce the release of TNF-α and IL-4 and to block the recruitment of neutrophils into tissues as well as the production of LTB4. However, to date no studies have evaluated the effects of cilomilast on airway cells isolated from subjects with COPD. We have therefore undertaken a study to investigate the spontaneous release of TNF-α, IL-8, and GM-CSF by bronchial epithelial cells and by sputum cells isolated from normal subjects and smokers, with or without COPD. The anti-inflammatory properties of cilomilast were also examined by assessing its ability to inhibit the release of TNF-α, IL-8, and GM-CSF by bronchial epithelial cells and sputum cells. Furthermore, to obtain a better understanding of the potential activity of cilomilast on neutrophilic inflammation in COPD, we analysed the chemotactic activity of sputum cell and bronchial epithelial cell supernatants recovered from cultured cells in the presence or absence of cilomilast.

**METHODS**

**Patients**
The study was performed on three groups of subjects: 10 smokers with COPD (median age 63 years, range 52–74, 25–75% percentiles 56–71), 10 smokers without COPD (healthy smokers) (median age 41 years, range 33–76, 25–75% percentiles 56–71), and 14 control subjects (median age 50 years, range 22–77, 25–75% percentiles 40–70).

COPD was defined and classified according to the criteria reported by GOLD guidelines. All subjects were smokers or ex-smokers (median 37.5 pack years, range 32.5–52). All...
COPD patients with radiographic or CT evidence of emphysema were excluded. Healthy smokers (median 40 pack years, range 32–45) had pulmonary function within the normal range. Normal subjects (controls) had never suffered from asthma or chronic bronchitis or from any bronchial or respiratory tract infection during the month preceding the study. All were lifelong non-smokers and their pulmonary function was within the normal range.

Subjects were excluded if they had had a bronchial infection during the month preceding the study. No subject had received corticosteroids or theophylline in any form during the 2 months before the study. The study was approved by the local ethics committee and subjects gave their informed consent.

Isolation of bronchial epithelial cells
Bronchial epithelial cells were recovered by bronchial brushing as previously described.20 Most of the patients were volunteers; two underwent bronchoscopy for diagnostic purposes. Briefly, the cells were pelleted by centrifugation (400g, 4°C, 10 minutes), washed three times, and resuspended in 1 ml RPMI at a final density of 1 × 10^6 cells/ml. Slides prepared by cytospin centrifugation (800g, 10 minutes, Cytospin, Shandon Southern Products, Runcorn, Cheshire, UK) of 200 µl of the cell suspension were stained with May-Grunwald-Giemsa for differential cell counts and by immunocytochemistry for cytokeratin using an alkaline phosphatase, anti-alkaline phosphatase (APAAP) system (Dako, Versailles, France) to assess their epithelial origin. Negative controls were prepared using a mouse IgG1 MOPC cell line antibody (Organon Laboratories, West Chester, PA, USA) as the primary antibody.

Treatment of bronchial epithelial cells with cilomilast
Bronchial epithelial cells were cultured in the absence or presence of cilomilast (SB-207499 BULK, SmithKline Beecham, UK) 1 µM for 24 hours at 37°C in a humidified 5% CO₂. This concentration of cilomilast was determined from three consecutive dose-response curves in which the effects of three concentrations of the drug (0.1, 1 and 10 µM) on the release of TNF-α and GM-CSF were assessed. Dose-response curve experiments with a higher number of concentration points were precluded by the limited number of cells. At the end of the incubation time the cell viability was >85%, as assessed by trypan blue exclusion, and supernatants were harvested and stored at -80°C for further analysis (TNF-α, IL-8, and GM-CSF) and chemotaxis assay.

Sputum induction and processing
Sputum induction and processing were performed according to the methods of Hargreave et al with modification.21 Differential cell counts were performed on cytocentrifuge preparations stained with May-Grunwald-Giemsa. In all cases 400 cells were counted by two blind observers and the results are expressed as a percentage of the total cells.

Treatment of sputum cells with cilomilast
Sputum cells were resuspended at a concentration of 1 × 10^6/ml in RPMI 1640 + 10% heat inactivated (56°C, 30 minutes) FCS + 1% penicillin-streptomycin solution + 1 mmol/l 1-glutamine (RPMI buffer). The sputum cells were cultured in the absence or presence of cilomilast 1 µM for 24 hours at 37°C in a humidified 5% CO₂. This concentration of cilomilast was determined on the basis of three consecutive dose-response curves in which the effects of three concentrations of the drug (0.1, 1 and 10 µM) on the release of TNF-α and GM-CSF were assessed. Dose-response curve experiments with a higher number of concentration points were precluded by the limited number of cells. At the end of the incubation time the cell viability was >85%, as assessed by trypan blue exclusion, and supernatants were harvested and stored at -80°C for further analysis (TNF-α, IL-8, and GM-CSF) and chemotaxis assay.

Table 1: Assessment of inhibitory effects of different concentrations of cilomilast on mediators released by bronchial epithelial cells and sputum cells isolated from patients with COPD

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Cilomilast 0.1 µM</th>
<th>Cilomilast 1 µM</th>
<th>Cilomilast 10 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>166 (165.2–201.2)</td>
<td>150 (138.7–161.2)</td>
<td>125.7 (111.3–131.9)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Results are expressed as median and 25–75th percentiles of three consecutive experiments. NA=not available because of undetectable levels of GM-CSF.
chemotactic activity of the supernatants was also evaluated and the results expressed as medians with 25–75th percentiles. The number of cells migrating spontaneously in the presence of RPMI 10% FCS under the same conditions as the supernatants (negative control) was subtracted from all measurements before data analysis.

Statistical analysis
The results are expressed as median values with 25–75th percentiles. Statistical analysis was performed with a non-parametric Kruskal-Wallis test with Bonferroni Dunn’s correction to assess differences between groups. Statistical analysis of the effects of bronchial epithelial and sputum cell supernatants cultured in the presence or absence of cilomilast was performed using the Wilcoxon test.

RESULTS
Demographic characteristics of patients
The differences in the median ages of the controls, healthy smokers, and subjects with COPD were not statistically significant. Forced expiratory volume in 1 second (FEV₁) was lower in patients with COPD than in smokers (p<0.0001; table 2). All patients with COPD had moderate disease (FEV₁ 43–75% predicted, median 44%, 25–75th percentiles 43–45%).

Total and differential cell counts in sputum
The viability of sputum cells was similar in the three study groups. The percentage of squamous cells in sputum samples was not significantly different between controls and smokers, or between smokers and patients with COPD. The median total cell count was higher in COPD patients (9.7 × 10⁶/g sputum, 25–75th percentiles 2.5–17.2), than in control subjects (2.4 × 10⁶, 25–75th percentiles 1.9–3.0; p=0.0023, table 3). The percentage of macrophages was significantly higher in control subjects and smokers than in patients with COPD (p=0.0001 and p=0.0002, respectively); the percentage of neutrophils was significantly higher in patients with COPD than in controls and smokers (p<0.0001 and p=0.0005, respectively); and the percentage of eosinophils was higher in patients with COPD than in controls (p=0.008). No statistical differences were observed in lymphocytes and bronchial epithelial cells (table 3).

Effects of cilomilast on TNF-α, IL-8, and GM-CSF release
Cultured bronchial epithelial cells isolated from patients with COPD released higher levels of TNF-α (165 (89–180) pg/ml) than cells isolated from smokers (65 (50–85) pg/ml) and control subjects (17 (12–25) pg/ml; p<0.0001). In addition, bronchial epithelial cells isolated from smokers with COPD released higher levels of TNF-α than those isolated from controls (p<0.0001; fig 1A). When bronchial epithelial cells isolated from COPD patients were cultured in the presence of cilomilast there was a significant inhibition of TNF-α release (116 (40–134) pg/ml; p=0.005; fig 1B).

Cultured sputum cells isolated from patients with COPD released higher levels of TNF-α than sputum cells isolated from smokers (404 (320–500) pg/ml) and control subjects (97.5 (90–115) pg/ml; p<0.0001). In addition, sputum cells isolated from smokers released higher levels of TNF-α than cells isolated from controls (p<0.0001; fig 1C). Furthermore, when the sputum cells isolated from COPD patients were cultured in the presence of cilomilast there was a significant inhibition of TNF-α release (1230 (1020–1230) pg/ml; p=0.005, fig 1D).

Cultured bronchial epithelial and sputum cells isolated from patients with COPD released higher levels of IL-8 than cells isolated from smokers and controls (bronchial epithelial cells: 4423.5 (4325–4589) pg/ml; p<0.0001, fig 2A; sputum cells: 4438.5 (4255–4677) pg/ml; p<0.0001, fig 2C).

Cilomilast did not significantly reduce IL-8 release by bronchial epithelial cells (baseline: 4423.6 (4235–4589) pg/ml; cilomilast: 4336.5 (4267–4526) pg/ml; % inhibition compared

Table 2

<table>
<thead>
<tr>
<th>Demographic characteristic of patients</th>
<th>Control (C)</th>
<th>Smoker (S)</th>
<th>COPD</th>
<th>p value†</th>
<th>C v S*</th>
<th>C v COPD*</th>
<th>S v COPD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>8</td>
<td>10</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex [M/F]</td>
<td>9/5</td>
<td>6/2</td>
<td>6/4</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)*</td>
<td>50 (40–70)</td>
<td>41 (33–59)</td>
<td>63 (56–71)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Smoke (% of current smokers)</td>
<td>0%</td>
<td>100%</td>
<td>70%</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Packs/year*</td>
<td>0</td>
<td>40 (32–45)</td>
<td>37.5 (32.5–52)</td>
<td>0.0001</td>
<td>p=0.009</td>
<td>p=0.0001</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>105 (100–110)</td>
<td>93 (90–103)</td>
<td>54 (50–62.5)</td>
<td>0.0001</td>
<td>p=0.009</td>
<td>p=0.0001</td>
<td>p=0.0001</td>
</tr>
</tbody>
</table>

Results are expressed as median with 25–75th percentiles in parentheses.

Table 3

<table>
<thead>
<tr>
<th>Cell analysis of sputum samples</th>
<th>Control (C)</th>
<th>Smoker (S)</th>
<th>COPD</th>
<th>p value†</th>
<th>C v S*</th>
<th>C v COPD*</th>
<th>S v COPD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability (%)</td>
<td>86.8 (67–89.3)</td>
<td>75.7 (73.4–83.3)</td>
<td>80.5 (74.6–87.8)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Squamous cells (%)</td>
<td>43 (2.9–8.0)</td>
<td>28 (1.1–4.0)</td>
<td>41 (1.2–5.4)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Total cell counts /10⁶/g sputum</td>
<td>2.4 (1.9–3.0)</td>
<td>5 (4.2–6.4)</td>
<td>9.7 (2.5–17.2)</td>
<td>NS</td>
<td>NS</td>
<td>p=0.0023</td>
<td>NS</td>
</tr>
<tr>
<td>Differential cell counts (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>83.1 (72.4–87)</td>
<td>57.2 (49.4–72.4)</td>
<td>25.2 (15.1–39.3)</td>
<td>p&lt;0.0001</td>
<td>NS</td>
<td>p&lt;0.0001</td>
<td>p=0.0002</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>15.1 (12.7–26.0)</td>
<td>40.6 (25.8–47.0)</td>
<td>68.2 (54.4–81.0)</td>
<td>p=0.0001</td>
<td>NS</td>
<td>p&lt;0.0001</td>
<td>p=0.0005</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.8 (0–1)</td>
<td>0.9 (0.3–1.2)</td>
<td>1 (0–1.7)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0 (0–0.3)</td>
<td>0.9 (0.1–1.7)</td>
<td>1.9 (0.8–4.8)</td>
<td>p&lt;0.008</td>
<td>NS</td>
<td>p=0.008</td>
<td>NS</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>0.4 (0.1–0.8)</td>
<td>0 (0–0.2)</td>
<td>0.2 (0–0.9)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as median with 25–75th percentiles in parentheses.
†p value for non-parametric Bonferroni Dunn’s post hoc test.
‡p value for non-parametric Kruskal-Wallis test comparing the differences between the groups.
From sputum cells incubated with cilomilast had significantly less chemotactic activity for neutrophils than supernatants from cells untreated with cilomilast (200.5 (150–221) pg/ml; p=0.005, fig 3B). Furthermore, cilomilast significantly inhibited GM-CSF release by sputum cells isolated from patients with COPD (322 (294–325) pg/ml) and control subjects (125 (100–150) pg/ml; p=0.002 and p=0.0001, respectively; fig 3A). Furthermore, cilomilast significantly inhibited GM-CSF release by sputum cells isolated from patients with COPD (322 (250–350) pg/ml; p=0.005, fig 3B).

**Effect of cilomilast on neutrophil chemotaxis assay**

Supernatant fluids harvested from bronchial epithelial and sputum cells from patients with COPD recovered and cultured in the presence or absence of cilomilast were tested for their capacity to induce neutrophil chemotaxis. Supernatants recovered from bronchial epithelial cells incubated with cilomilast had significantly less chemotactic activity for neutrophils than supernatants from cells untreated with cilomilast (200.5 (150–221) v 130 (120–169) cells high power field; p=0.008, fig 4A). Furthermore, supernatants recovered from sputum cells incubated with cilomilast had significantly lower chemotactic activity for neutrophils than those from cells untreated with cilomilast (198.5 (167–267) v 124 (119–172.5) cells high power field; p=0.006, fig 4B).

In addition, blocking experiments showed that the combination of an anti-IL-8 antibody with cilomilast exerted a greater inhibitory effect on neutrophil chemotaxis than either the anti-IL-8 antibody or cilomilast alone (from 80 (75–115) to 149 (127.5–200) or 124 (119–172.5) cells high power field).

**DISCUSSION**

This study shows that cilomilast inhibits the release of TNF-α and GM-CSF by bronchial epithelial cells and sputum cells isolated from patients with COPD. In addition, cilomilast significantly reduced the chemotactic activity of supernatant fluids harvested from cultured bronchial epithelial and sputum cells of COPD patients.

The levels of cAMP play an important role in the modulation of the functional activation of several airway cells. Increased levels of cAMP have been shown to decrease the release of neutrophil chemotactic activity as well as neutrophil adhesion to bronchial epithelial cells via MAC-1. In airway epithelial cells the rise in cAMP was found to be protective against oxidants and to enhance the release of prostaglandin E2. It is therefore conceivable that increases in the cellular concentration of cAMP not only mediate the relaxation of airway smooth muscle but also inhibit the activation of inflammatory cells.

Inflammatory and immunomodulatory cells contain PDE4 and many functions of these cells are inhibited by selective PDE4 inhibitors. In addition, PDE4 seems to be the most important isoenzyme in both the microsomal and cytoplasmic compartments of airway cells.

Cilomilast is a second generation PDE4 inhibitor effective in the treatment of COPD. It has high selectivity for the cyclic AMP specific (or PDE4) isoenzyme. In vitro, cilomilast suppresses the activity of many proinflammatory and immune cells implicated in the pathogenesis of COPD and is highly active in animal models. In vitro studies have also suggested that cilomilast can inhibit antigen driven IL-5 production, cytokine induced adhesion to endothelial cells, and chemotaxis. However, most of these effects have been evaluated using cell lines or animal models and, to date, no studies have assessed the effects of cilomilast on airway cells isolated from patients with COPD.
In this study sputum cells were cultured to assess their ability to release inflammatory mediators and to respond to a drug such as cilomilast. It should be noted that, in reality, drugs or stimuli usually target the various cell types present in the airways and rarely just a single cell population, so we used airway cells from COPD patients to confirm the ability of cilomilast in a concentration of 1 µM to influence the functional activation of airway cells. This concentration of cilomilast was chosen based on the results of the dose-response curve experiments as well as on previous evidence. Cilomilast significantly inhibited the release of TNF-α and GM-CSF by both epithelial and sputum cells, while there was no inhibitory effect on IL-8 release.

It is unlikely that the heterogeneity of the cell population is the main cause of the different effects of cilomilast on mediator release and on the low or absent inhibitory effect on IL-8 as similar results were obtained with cultured bronchial epithelial cells which were virtually pure. There are several possible explanations for the lack of inhibition by cilomilast on IL-8 release. It is possible that IL-8 release might have been inhibited by a higher concentration or a different incubation time. In addition, as IL-8 release by airway cells is regulated by complex intracellular pathways, it is possible that some of them may not be targeted by cilomilast. This hypothesis is supported by a previous study which showed that enhanced cAMP levels in epithelial cells did not inhibit IL-8 release, suggesting that IL-8 release is not solely modulated by cAMP. Moreover, the extent of the inhibitory effect of cilomilast may differ according to the clinical severity of COPD—being greater in patients with severe COPD than in those with mild COPD in whom treatment with theophylline reduced sputum

Figure 2  IL-8 release by (A) bronchial epithelial cells (BEC) and (C) sputum cells isolated from the airways of controls, smokers, and patients with COPD. The recovered bronchial epithelial cells and sputum cells were cultured for 24 hours at 37°C to test the release of IL-8 either spontaneously or after incubation with cilomilast 1 µM (B and D). Statistical analysis was performed by the Mann-Whitney U test for comparisons between groups and by the Wilcoxon U test for comparisons between baseline and cilomilast.

Figure 3 GM-CSF release by sputum cells (A) isolated from the airways of controls, smokers, and patients with COPD. The recovered sputum cells were cultured for 24 hours at 37°C in the presence or absence of cilomilast 1 µM (B). Statistical analysis was performed by the Mann-Whitney U test for comparisons between groups and by the Wilcoxon U test for comparisons between baseline and cilomilast.
levels of IL-8 by 24% compared with baseline.23 However, Culpitt et al24 assessed the effects of theophylline on IL-8 levels in sputum supernatants obtained immediately after sputum processing while we evaluated the effects of cilomilast on IL-8 release from sputum cells cultured for 24 hours. Whether this effect reflects different modes of action of theophylline and cilomilast or whether it depends on the different profile of airway inflammation in moderate and severe COPD needs to be studied further.

The findings of this study enable us to define better the scientific rationale for the use of cilomilast in the treatment of COPD, and provide important information about the mechanisms by which it may affect the inflammatory process in the airways of these subjects. Interestingly, the inhibitory effect of cilomilast on TNF-α and GM-CSF release by airway cells was associated with a significant reduction in neutrophil chemotactic activity exerted by supernatants harvested from cultured bronchial epithelial cells and sputum cells incubated for 24 hours with the drug. It is also likely that cilomilast may exert a direct inhibitory effect on neutrophil chemotaxis, as has already been demonstrated on fibroblast migration.25 It is of note that, although significant, the inhibitory effect on neutrophil chemotaxis was not complete. This might be attributed to the low inhibitory effect of cilomilast on IL-8 release by both bronchial epithelial and sputum cells, as well as to the presence of other chemotactic mediators for neutrophils such as LTB4.

Taken together, the results of the present study suggest that cilomilast has the potential to inhibit the development of neutrophilic inflammation in the airways of patients with COPD. Indeed, it is believed that neutrophil recruitment and activation is an important step in the pathogenesis of airway inflammation in this disease, as shown by the increased numbers of neutrophils in both central and peripheral airways as well as by their distribution in the epithelial layer and within mucus glands. It is well known that neutrophil activation leads to the release of a wide variety of inflammatory mediators such as proteases and oxygen free radicals26 that not only amplify the recruitment of inflammatory cells, but also cause damage to lung tissues.

It is therefore conceivable that drugs capable of inhibiting neutrophil recruitment and function may be a promising strategy in the treatment of COPD for several reasons. Firstly, so far very few drugs have been shown to inhibit neutrophil function as well as the release of mediators by airway cells isolated from patients with COPD.27 Secondly, the effects of cilomilast on the release of inflammatory mediators further support the concept that this drug not only provides bronchodilatory effects but is also characterised by anti-inflammatory properties which appear to be effective on neutrophils. Thirdly, the inhibitory effects of cilomilast on the release of neutrophil chemotactants by bronchial and sputum cells isolated from patients with COPD suggest that these cells have a certain degree of responsiveness to this drug, a finding which has not always been observed with other drugs including corticosteroids.28

This study has indicated several anti-inflammatory effects of cilomilast on airway cells isolated from patients with COPD and supports its potential usefulness in the treatment of this disease.

Authors’ affiliations
V Bellia, A M Vignola, Istituto di Medicina Generale e Pneumologia, Università di Palermo, Italy;
M Profita, G Chiappara, F Mirabella, R Di Giorgi, L Chimenti, G Costanzo, L Riccobono, A M Vignola, Istituto di Fisiopatologia Respiratoria, CNR, Via Trabucco, Palermo, Italy;
J Bousquet, INSERM, U-454 Montpellier, France.

This study was supported by GlaxoSmithKline USA and by INSERM-CNR.

REFERENCES
Contamination of rhinovirus stocks with poliovirus type 1

One of the major successes of public health vaccination programmes has been the eradication of circulating poliovirus from the majority of countries in the world. Reintroduction of wild type poliovirus may occur following spread from sources of virus stored in virological and other laboratories for research purposes or from countries where polio remains endemic. It is therefore of obvious concern that the authors at the National Collection of Pathogenic Viruses (NCPV), whilst characterising stocks of rhinovirus from the MRC Common Cold Research Unit, found that five of 22 cell cultures had abnormal plaques and were confirmed to contain poliovirus type 1 with likely contamination of two second generation rhinovirus stocks. It is therefore of obvious concern that the authors at the National Collection of Pathogenic Viruses (NCPV), whilst characterising stocks of rhinovirus from the MRC Common Cold Research Unit, found that five of 22 cell cultures had abnormal plaques and were confirmed to contain poliovirus type 1 with likely contamination of two second generation rhinovirus stocks.