Bronchial epithelial cells cultured from clinically stable lung allograft patients promote the development of macrophages from monocytes rather than dendritic cells.

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Abstract

Introduction: It is understood that chronic allograft failure occurs as a result of alloimmune and non-allo-immune injury. Dendritic cells (DC) are thought to be crucial in regulating (allo)immune airway damage and interactions with epithelial cells are likely. Studies in human lung transplantation are limited however, and the available literature on DC inconsistent. Our study focused on the ex-vivo influence of primary bronchial epithelial cells derived from lung allografts on DC differentiation.

Methods: Epithelial cell conditioned media (ECCM) were added to monocytes differentiating into DC under the influence of Interleukin-4 and Granulocyte Macrophage-Colony Stimulating Factor. The resultant cells were compared to DC cultured without ECCM and to monocyte-derived macrophages. Expression of typical DC (e.g. CD1a) and macrophage (e.g. CD14) markers was assessed by flow cytometry. Phenotypical assessments were complimented by functional studies of mannose receptor-mediated phagocytosis (FITC dextran uptake) and antigen-presenting capability (Mixed Lymphocyte Reactions).

Results: Cells exposed to ECCM expressed statistically significantly lower levels of CD1a than unexposed DC. CD14 expression was increased, as was phagocytic function. ECCM-cultured cells also expressed lower levels of T-cell co-stimulatory molecules, secreted an anti-inflammatory cytokine profile and had significantly reduced antigen-presenting capability.

Conclusion: Using phenotypic and functional approaches our study has shown that ECCM from lung allografts drives the production of macrophage-like cells from monocytes, rather than DC. Our data suggest that epithelial cells may restrain airway DC and potential alloimmunity. It remains unclear whether the observed effect is specifically seen in lung transplant recipients or might be a general property of bronchial epithelial cells. It is possible this may reflect a homeostatic inter-relationship between airway epithelial and DC populations relevant both to lung allografts and the lung more generally.

Key Words: Lung Transplantation, Dendritic cells, Bronchial epithelial cells.
Introduction

Lung transplantation is an accepted treatment for end stage lung disease in carefully selected patients but success is compromised by chronic allograft dysfunction.

Episodes of acute rejection are consistently identified as a key risk factor for chronic allograft dysfunction in International registry data, and this devastating complication is referred to as chronic rejection, reflecting the current understanding that chronic alloimmune damage is a key mechanism. Dendritic cells (DC) are the professional antigen presenting cells of the airway that initiate and regulate immune responses and DC are thought to be crucial in regulating (allo)immune damage in man. Studies in human lung transplantation are limited however, and the available literature is inconsistent.

Yousem et al showed increased numbers of DC in chronically rejecting lung allografts and a subsequent study by Leonard et al indicated that DC co-expressing the co-stimulatory molecules CD80 and CD86 were more frequent in allografts with chronic rejection. In contrast a previous study from our centre showed that there were fewer CD1a positive DC in lung allografts compared to normal lungs.

The airway epithelium is a recognised focus of damage in chronic allograft dysfunction but it is also increasingly accepted that airway epithelial cells may play a key part as effector cells, through the production of a wide range of inflammatory and immunomodulatory cytokines and growth factors. Physiologically airway DC are thought to continuously sample the airway lumen for potential pathogens by projecting dendrites through the airway epithelium and this close spatial arrangement emphasises the potential for epithelial cells to interact with, and modulate, DC function. Regamey et al have recently studied this potential using conditioned media produced from alveolar and epithelial cell lines. They showed that epithelial cells, both constitutively and following stimulation with pro-inflammatory cytokines such as TNFα, drove the production of DC from monocytes in an interleukin (IL)-15 dependent manner. IL-15 is a cytokine that shares biological properties with IL-2, and recombinant IL-15 has been shown to cause the production of DC from monocytes. A potential implication of this study is that in lung transplantation, airway epithelial cells may promote a situation which is deleterious to the graft, with an expanded population of DC capable of orchestrating alloimmune injury.

To our knowledge there are no studies in primary epithelial cell cultures from lung allografts evaluating the potential for epithelial cells to modulate the production of dendritic cells from monocytes. In this study, recognising the lack of specificity of phenotypic markers alone, we have investigated phenotypic and functional properties for DC using a previously validated ex-vivo DC culture system. Using a coordinated and multi parametric approach we tested the hypothesis that epithelial cell conditioned media can modulate phenotypic and functional properties of DC.
Methods (additional methods are available as an online supplement)

Ethics
The study was approved by the Local Research Ethics Committee and informed consent obtained from all participants.

Transplant patient clinical data
Clinically stable lung allograft recipients free from chronic allograft dysfunction were used to obtain primary epithelial cells. Clinical details are summarised in Table 1.

Bronchoscopy and Bronchial Sampling
Our post transplant patients undergo surveillance bronchoscopy at one week, one month, three, six and twelve months as well as further bronchoscopy if indicated on clinical grounds. All patients underwent pulmonary function testing and an assessment of clinical status was made, based on standardised criteria. Bronchoscopy was performed in accordance with international guidelines.

Bronchoalveolar Lavage fluid (BAL) was obtained from either the lingula or right middle lobe and sent for routine microbiological testing. Bronchial brushings (n=4-6) were obtained from subsegmental bronchi using a standard single-sheathed nylon cytology brush (5 fr; Wilson-Cook) and dispersed in 5ml of Phosphate Buffered Saline (Sigma). Finally, transbronchial specimens were taken from either the right or left lower lobe and sent for histopathological examination to exclude acute vascular rejection based on standard ISHLT criteria.

Primary bronchial epithelial cell culture
Primary BECs were obtained from bronchial brushings as previously described. In brief, endobronchial brushings were plated in serum-free Bronchial Epithelial Basal Media (BEBM; Lonza) supplemented with single quots and antibiotics. When 70-90% confluent, cells were passaged using trypsin to T75 flasks or 24 well plates. When 80-90% confluent, 24 well plates were rested for 24 hours in serum-free BEBM media. Epithelial cell conditioned supernatants (ECCM) were then collected following 48 hours of culture. Microbiological contamination of ECCM was excluded by testing of ECCM for normal respiratory flora and fungal and bacterial pathogens in an independent, clinically accredited laboratory.

Generation of DC and macrophages from monocytes
Monocytes were isolated from freshly drawn peripheral blood from healthy donors or buffy coats, as described previously. Briefly, peripheral blood mononuclear cells were isolated by density centrifugation on Lymphoprep (Axis-Shield Diagnostics, Dundee, UK). CD14+ monocytes were isolated by positive magnetic selection using anti-CD14 magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocytes were cultured at 0.5x10^6 cells/ml for 6 days in 0.5 ml BEBM and 0.5 ml RPMI-1640 supplemented with 10% foetal bovine serum, 2mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. To generate DC, IL-4 and GM-CSF (50 ng/ml each, Immunotools, Friesoythe, Germany) and to generate macrophages (Mph), M-CSF (50 ng/ml, R&D Systems Europe Ltd,
Abingdon, U.K.) were added to the monocyte cultures on day 0 and day 3. After 6 days, cells were stimulated with LPS (100 ng/ml, Sigma, Poole, UK) or were left untreated. After 24 hours, supernatants were harvested and assayed for IL-12p70 and IL-10 by ELISA, and the DC and MPh were extensively washed before performing functional assays and flow cytometry.

**Effect of epithelial cell conditioned media (ECCM) on DC cultures**

Monocytes were cultured under DC-generating conditions as described above but with 0.5ml ECCM instead of BEBM. The ECCM-modified DC cultures are referred to as antigen-presenting cells (APC) throughout the paper.

**Flow Cytometry**

The following antibodies were used for cell surface marker analysis: CD1a (NA1/34; Dako, Glostrup, Denmark), CD14 (M5E2), CD68 (27-35), CD86 (2331 FUN-1), CD83 (Hb15e) and HLA-DR (L243; all from BD Pharmingen, San Jose, CA, USA). Isotype-matched control antibodies were used to confirm that staining was specific (data not shown). Cells were centrifuged and resuspended in FACS buffer (Phosphate buffered saline supplemented with 3% FCS, 2mM EDTA, and 0.01% sodium azide). Human IgG (Grifols, Los Angeles, CA, USA), was added with antibodies to prevent Fc receptor binding. To detect intracellular CD68 a permeabilisation step was included (Cytofix/cytoperm, BD Biosciences). Cells were incubated on ice for 30 min, washed and resuspended in FACS buffer. Data were collected on a Becton Dickinson FACScan and analysed using FlowJo (Treestar).

**Phagocytosis assay**

Fluorescent labelled dextran (FITC-dextran; Sigma) was used to assess the mannose receptor (MR)-mediated phagocytic capacity of the cell types. Cells were incubated with FITC-dextran at 37 °C for one hour, while a control group was left on ice to exclude extracellular binding of FITC-dextran. Cells were extensively washed and intracellular FITC-dextran was quantified by flow cytometry.

**Mixed Lymphocyte Reaction (MLR)**

1x10⁴ DC, APC or MPh were cultured with 1x10⁵ allogeneic CD3⁺ T lymphocytes in 200μl cultures in a 96-well plate. Supernatants were harvested after 6 days and assayed for IFN-γ by ELISA. Proliferation was assessed by incorporation of ³H-thymidine for the last 8 h of culture by scintillation counting (Microbeta TriLux, Perkin Elmer, USA).

**Enzyme-linked Immunosorbent Assay (ELISA)**

Cytokine levels in the supernatants were measured by specific sandwich ELISA, performed with commercially available matched antibody pairs. The following cytokines were measured IL-8 and IL-15 (R&D Systems), IFN-γ, IL-6, IL-10 and IL-12p70 (BD Pharmingen).

**Statistical analysis**

Statistical software used for the analysis was GraphPad Prism (version 4). In the upper panels of Figure 1 the medians of CD1a, CD14 and CD68 expression were compared by the non-parametric paired Wilcoxon test for the following comparator
group pairs: DC and APC; APC and MPh. In the lower panels of Figure 1, the
medians of CD86, CD83 and HLA-DR expression were compared by the non-
parametric paired Wilcoxon test for the following comparator group pairs: DC and
LPS-DC; APC and LPS-APC; MPh and LPS-MPh; LPS-DC and LPS-APC; LPS-
APC and LPS-MPh. Only significant results (p<0.05) are indicated in the Figure. In
Figure 2, the means of IL-10 and IL-12p70 production and the mean of Dextran
FITC uptake were compared by t-test for the following comparator group pairs: DC
and APC; APC and MPh. Only significant results (p<0.05) are indicated in the
Figure.
Results (additional results are available as an online supplement)

1. Bronchial epithelial cell-conditioned media favour differentiation of monocytes towards a macrophage phenotype at the expense of DC.

Table 2 summarises data showing that bronchial epithelial cell-conditioned media (ECCM) contained a mixture of secreted cytokines, including measurable levels of IL-8 and IL-6. IL-15 was not detectable in any of the epithelial cell cultures.

Figure 1 shows the expression of markers by the 3 monocyte-derived cell populations: i) DC (generated with IL-4 and GM-CSF); ii) antigen-presenting cells (APC) cultured by adding ECCM to the IL-4/GM-CSF DC cultures and iii) macrophages (MPh: generated with M-CSF). The effect of a further 24-hour stimulation with LPS was assessed for the above cell populations. This is known to activate immature DC, resulting in the upregulation of markers involved in T cell activation.

Phenotypic markers assessed were the DC marker CD1a, the MPh markers CD14 and CD68, the antigen presenting molecule HLA-DR, the classical DC maturation marker CD83, and the T cell co-stimulatory molecule CD86. The salient findings of these experiments showed that ECCM drove the production of MPh-like cells from monocytes. Within the APC population there were significantly less cells expressing the DC marker CD1a compared to the DC population, contrasting with significantly more cells expressing the MPh marker CD14. APC also expressed higher levels of CD68 but this was not statistically significant.

Introduction of a LPS stimulation to the protocol led to a significant up-regulation of HLA-DR; CD83 and CD86 on both DC and APC, but not MPh. However, the levels of CD83 and CD86 were significantly lower on LPS-activated APC than on LPS-activated DC, whilst expression levels of HLA-DR were similar.

We also measured the production of the anti- and pro-inflammatory cytokines IL-10 and IL-12p70, respectively, by DC, APC and MPh that had been activated by LPS for 24 h (Figure 2A). APC produced significantly higher levels of IL-10 and lower levels of IL-12p70 than DC, again resembling MPh rather than DC. Non-stimulated cell populations did not secrete detectable levels of cytokines (data not shown).

2. Epithelial cell-conditioned media induce APC that functionally resemble MPh rather than DC.

Because the phenotype of APC resembled the phenotype of MPh rather than DC, we hypothesised that the functional characteristics of APC would also be more macrophage-like. To test this hypothesis we assessed functional abilities that are typical for either MPh (phagocytosis) or DC (T cell stimulation).

Figure 2B depicts an assay of mannose receptor (MR)-mediated phagocyte function. Here the MPh represent a positive control as professional phagocytes.
Like MPh, ECCM-induced APC exerted greater MR-mediated phagocytic activity compared to DC.

Figure 3 summarises the results of mixed lymphocyte reactions to assess the immunostimulatory capacity of the monocyte-derived populations. Here LPS-activated DC represent a positive control as professional antigen-presenting cells, potently initiating T cells responses. Activation of DC with LPS indeed resulted in enhancement of T cell stimulatory activity of DC, with higher induction of proliferation and production of the prototypic cytokine IFN-γ by allogeneic T cells. This enhanced T cell stimulatory capacity of LPS-DC is consistent with the higher expression of CD83 and CD86 and the production of the Th1-inducing cytokine IL-12p70. In contrast, activation of APC with LPS did not enhance their immunostimulatory capacity and especially the induction of IFN-γ production was greatly reduced as compared to DC and LPS-DC. MPh were the poorest stimulators of T cell proliferation and only induced very low levels of IFN-γ. These experiments show that ECCM-induced APC have a low immunostimulatory capacity compared to DC.

Together these data indicate that ECCM skew monocyte-derived DC towards a macrophage-like population in terms of phenotype and function.
Discussion
This study has shown that conditioned media from lung allograft primary bronchial epithelial cells drove the production of macrophage-like cells from monocytes, rather than DC. Although there was biological variability, a broad range of phenotypic and functional markers consistently supported this finding. Our data suggest that epithelial cells from allograft recipients promote production of MPh, while restraining the airway DC population and potential alloimmunity. It remains unclear whether the observed effect is specifically seen in lung transplant recipients or might be a general property of bronchial epithelial cells. It remains to be studied whether this is relevant both to lung allografts and to the lung more generally.

The biology of professional antigen presenting cells in the airway is likely to be of key importance in the alloimmune component of chronic allograft dysfunction, which is commonly referred to as chronic rejection. There are few studies in lung transplantation, however, and these are restricted to phenotypic approaches, using different markers, which report conflicting data. Two studies indicate that in chronic rejection there are increased numbers of DC, with an earlier study from our group indicating lower numbers of CD1a positive DC in lung allografts.

It is accepted that the local environment to which DC are exposed is a key factor in their maturation and function. The close spatial relationship between airway epithelial cells and DC indicate that epithelial cells might be an important influence. Airway epithelial cells secrete a spectrum of pro-inflammatory cytokines and growth factors, which play an important role in local defence and in the induction of a systemic inflammatory response. In lung allograft primary cultures we have previously reported that airway epithelial cells constitutively secrete GM-CSF, M-CSF, and IL-6, factors that increase the production and mobilization of monocytes from the bone marrow and influx into the lung. In ex-vivo models, where DC are produced from monocytes, GM-CSF is used in the conditioning protocols, emphasising the potential for epithelial cell derived products to influence DC differentiation. Further research is required to define the epithelial cell-factor(s) interfering with DC differentiation. We considered the possibility that the anti-inflammatory cytokine IL-10 was involved, but could not detect this cytokine in ECCM by ELISA (lower limit of detection 15 pg/ml, data not shown). Another possibility is IL-6, which was detectable in the majority of the ECCM used in this study. It has been reported that high levels of IL-6 (>50 ng/ml) but not low levels of IL-6 (20 pg/ml) switches monocyte differentiation from dendritic cells to macrophages. Because ECCM contain only moderate levels of IL-6 (30-250 pg/ml; Table 2), we feel it is unlikely that IL-6 by itself was responsible for the observed inhibitory effects on DC differentiation, although it cannot be excluded that IL-6 synergised with other soluble ECCM component(s).

The potential for epithelial cells to interact with DC was recently studied by Regamey et al, using lung A549 and airway BEAS-2B epithelial cell lines. Conditioned media from the epithelial cells contained IL-15 both constitutively and following stimulation with inflammatory stimuli. Addition of these media to monocytes led to an up regulation of IL-15 receptor mRNA expression, with
differentiation of monocytes to functional DC\textsuperscript{9}. In the allograft setting, where upregulated alloimmunity is damaging\textsuperscript{1} \textsuperscript{16}, these findings are of potential significance, but there are no previous data that we are aware of in primary airway epithelial cells from allograft patients.

Our findings are not consistent with those of Regamey et al. Using accepted markers for DC and macrophages we consistently showed that conditioned media from allograft recipient primary epithelial cells favoured the production of macrophage-like cells from monocytes. Phenotypic markers were corroborated by functional phagocytic and immunostimulatory assays. Our data showed that incubation of monocytes with patient derived epithelial cell-conditioned media consistently yielded a population of cells with low expression of DC markers and poor T cell stimulatory ability. In contrast, we observed significant expression of macrophage markers and competent phagocyte function. The conditioned media from allograft primary epithelial cells did not contain detectable levels of IL-15 as measured by ELISA (data not shown), which may reflect differences between primary epithelial cells and cell lines and/or differences in the experimental set up (e.g. cell density).

A main difference between our work and the study by Regamey et al was that we used primary epithelial cells, sourced from lung transplant recipients. It is of interest that a previous study by Ohtoshi et al using upper airway epithelial cells from polyps drove the production of MPh from monocytes, although this was in atopic, but otherwise healthy volunteers and not a transplant related study investigating DC markers\textsuperscript{17}. More recent work involving an airway epithelial cell line, and murine DC has also indicated that epithelial cells may modify immune responses by inducing an “anti-inflammatory”, DC suppressing microenvironment\textsuperscript{18}. This may indicate that our data, which are the first such in primary human cells as far as we are aware have relevance beyond lung transplantation. Promoting the production of MPh-like cells is arguably physiologically appropriate in the human airway since MPh form an important defence in the constantly exposed lungs. The antimicrobial activity of MPh is promoted by efficient phagocytosis and together with the barrier function provided by the tight airway epithelium, MPh represent a first line of innate immunity\textsuperscript{19}.

Danger signals, provided by stimuli such as infection in the allograft may be important in promoting alloimmunity\textsuperscript{16}. The emerging paradigm is that non-alloimmune damage due to potentially occult challenges such as infection and aspiration may combine with alloimmunity to yield damage\textsuperscript{16}. The airway epithelium is a target for injury, with damage and loss of barrier function in chronic allograft dysfunction. Our recent finding of decreased levels of the epithelial cell product secretory leukocyte proteinase inhibitor (SLPI) in the BAL of lung allograft recipients with BOS support this model\textsuperscript{20}. In addition, our study showed that the antimicrobial peptide LL-37 is elevated in the BAL of allograft recipients with chronic dysfunction\textsuperscript{20}. As well as being an antimicrobial, LL-37 has been shown to interact with DC, and CD86 expression following LL-37 treatment has been shown to be increased in a dose dependent manner\textsuperscript{21}. 
In the present study we showed that monocytes incubated with ECCM and then stimulated with the Toll like receptor 4 agonist LPS, up-regulated HLA-DR, CD83 and CD86, which are markers of DC activation. However, the T cell stimulatory capacity of ECCM-induced APC was not enhanced. It is likely that the high production of the anti-inflammatory cytokine IL-10 by LPS-activated APC will have counteracted the T cell co-stimulatory activity of CD83 and CD86, but this requires further investigation. Interestingly, the ECCM-induced APC resemble regulatory DC (e.g. high IL-10 production, low T cell stimulatory capacity) described previously by us and others \(^{14 22 23}\) but whether they are capable of inducing T cell tolerance would require further investigation.

Our data emphasise the need for primary cell data to be assessed along with experiments involving cell lines, although primary studies are themselves limited by technical difficulty and the fact that they are resource intensive. Further studies would be useful to investigate conditioned media sourced from epithelial cells from subjects with chronic allograft dysfunction. This was beyond the scope of this present study, since our experience is that such cultures commonly fail, due to antibiotic resistant patient derived infection\(^{13}\). Such work may require specialised protocols and augmented, possibly patient specific, antibiotic approaches.

Our experiments were all performed using epithelial cells at early passage (I-II), in cell cultures free from transplant maintenance therapies. We do not expect previous patient immunosuppression to be responsible for our findings but transplant medications might affect the interplay between epithelial cells and DC in the airway. This could also be an avenue for further research. An aim of this could be to elucidate the most appropriate balance between appropriate immunosupression and preservation of airway homeostasis, with maintenance of epithelial function and appropriate networking with airway DC representing a desirable goal.
Legends

Table 1. Clinical details for patients from whom primary epithelial cell cultures were derived.

Table 2. Summary of IL-8 and IL-6 measurements made from ECCM.

Figure 1. Phenotype of monocyte-derived cell populations. Expression of markers by DC, APC and MPh was assessed by flow cytometry. Lower panels include cell populations activated with LPS for 24 h. Debris and dead cells were excluded on the basis of forward-scatter and side-scatter. Data are shown of at least 6 independent experiments; different epithelial cell donors and blood donors were used in each experiment. Horizontal lines represent median values. * p<0.05 as determined by the Wilcoxon test.

Figure 2. Cytokine production and MR-mediated phagocytic capacity of monocyte-derived cell populations. (A) Cytokine production. DC, APC and MPh were activated for 24 h with LPS (100 ng/ml). Levels of IL-10 and IL-12p70 were determined by specific sandwich ELISA. The mean production +/- SEM of 6 independent experiments is shown. (B) Phagocytic activity. Uptake of FITC-dextran by DC, APC and MPh after 1 h was assessed by flow cytometry. Data are shown as the mean +/- SEM of at least 3 independent experiments. Different epithelial cell donors and blood donors were used in each experiment. * p<0.05 as determined by the t-test.

Figure 3. Immunostimulatory capacity of monocyte-derived cell populations. DC, APC and MPh were or were not activated with LPS for 24 h and were used as stimulator cells in a mixed lymphocyte reaction with allogeneic CD3+ T cells. After 6 days, T cell proliferation was determined by 3H-Thymidine incorporation and IFN-γ production by specific sandwich ELISA. Results are representative of 3 independent experiments and are shown as mean +/- SEM. Different epithelial cell donors and blood donors were used in each experiment.
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<th>Patient Sample</th>
<th>Time post-transplant</th>
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<th>Transplant</th>
<th>Age at time of transplant</th>
<th>Biopsy</th>
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<th>BAL Microbiology</th>
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Key:
SL = Single lung, HL = Heart lung
Biopsy = TBB scored as per international guidelines\(^\text{12}\) for acute and chronic rejection
BOS Score= Bronchiolitis Obliterans Score\(^\text{10}\)
### TABLE 2

<table>
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<th>Patient ECCM Sample</th>
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REFERENCES


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Introduction

In addition to the data presented in the main manuscript, we have additional primary cell culture experiments, which address the question of how conditioned media from epithelial cells interact with monocytes.

In a previous study by Regamey at al, supernatants from epithelial cell lines drove the differentiation of DC from monocytes (1). We have therefore evaluated whether ECCM supernatants from primary bronchial epithelial cells could also drive the differentiation of DC from monocytes.

Methods

Experiment 1
ECCM were derived from 48 h primary epithelial cell cultures that were left untreated (ECCM) or were treated with IL-17 (ECCM-IL-17). Monocytes were isolated from peripheral blood by positive selection with CD14 magnetic beads and were cultured for 6 days in absence (Monocytes) or presence of IL-4/GM-CSF (mo-DC), ECCM, ECCM-IL-17, or rIL-17. After 6 days cell death was determined by incorporation of Trypan Blue. The results are representative of 3 independent experiments.

Experiment 2
ECCM were derived from 48 h primary epithelial cell cultures. Monocytes were isolated from peripheral blood by positive selection with CD14 magnetic beads and were cultured for 6 days in absence (Monocytes) or presence of IL-4/GM-CSF (mo-DC) or ECCM. After 6 days expression of CD1a (A) and CD14 (B) was determined by flow cytometry. Dead cells and debris were excluded from the analysis by gating on live cells in the forward scatter/side scatter plots. The results are presented as mean +/- SEM of 3 independent experiments.

Results

The results are summarised in Fig 1 and 2.

Our supplemental data showed that ECCM did not sustain monocyte survival in a 6-day culture period, suggesting that ECCM from primary cells did not contain sufficient growth factors (Figure 1 below).
When we analysed the phenotype of the surviving cells (appr. 10%), we found that some degree of DC differentiation had taken place, as assessed by the enhanced expression of CD1a, the classical monocyte-derived DC marker (Figure 2A below) and enhanced size and granularity of the cells (data not shown). However, CD14 expression was not decreased in these cultures, while monocyte-derived DC are characterized by low CD14 expression (Figure 2B below).

Discussion

Our results suggest that ECCM from primary cells may, to some degree, drive DC differentiation. However the high level of cell death in the 6-day monocyte cultures made it difficult to analyse these cells. We conclude that the system was not sufficiently robust for pursuing these types of experiments in the primary cell we were studying.

Reference

Figure 1. ECCM do not sustain monocyte survival. ECCM were derived from 48 h primary epithelial cell cultures that were left untreated (ECCM) or were treated with IL-17 (ECCM-IL-17). Monocytes were isolated from peripheral blood by positive selection with CD14 magnetic beads and were cultured for 6 days in absence (Monocytes) or presence of IL-4/GM-CSF (mo-DC), ECCM, ECCM-IL-17, or rIL-17. After 6 days cell death was determined by incorporation of Trypan Blue. The results are representative of 3 independent experiments.

Figure 2. Enhanced CD1a expression on monocytes cultured in ECCM. ECCM were derived from 48 h primary epithelial cell cultures. Monocytes were isolated from peripheral blood by positive selection with CD14 magnetic beads and were cultured for 6 days in absence (Monocytes) or presence of IL-4/GM-CSF (mo-DC) or ECCM. After 6 days expression of CD1a (A) and CD14 (B) was determined by flow cytometry. Dead cells and debris were excluded from the analysis by gating on live cells in the forward scatter/side scatter plots. The results are presented as mean +/- SEM of 3 independent experiments.
Figure 1
Figure 2
Figure 3
Bronchial epithelial cells cultured from clinically stable lung allograft patients promote the development of macrophages from monocytes rather than dendritic cells.

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