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Evidence for an Immunological Role of Mouse Primary Lung Epithelial Cells

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ABSTRACT

The present mini-review covers our recent work on isolation of mouse primary lung epithelial (PLE) cells and morphological and phenotypic changes of these cells in culture. With regards to the markers that define type I and type II PLE cells, we find that the freshly isolated PLE cells from mouse lungs have a mixed phenotype but in culture, the proportion of type I epithelial cells increases to about 95% whereas type II cells decline to <1%. We further provide evidence for PLE cells to have a role in lung immunity. PLE cells express TLR2 and 4 receptors that can be up-regulated by exposure to BCG antigens. In culture, PLE cells secrete TNFα when exposed to BCG or LPS. We also show that PLE type I cells have the ability to process and present BCG antigens to sensitized T helper cells. Thus, PLE cells may participate in both innate as well as adaptive immune function in lungs.

Keywords: Lung epithelial cells, PLE cells, Podoplanin, CD74, TLR2, TLR4, BCG, T helper cells, TNFα, IL2, IFNγ, Innate and adaptive immune responses, Lung

INTRODUCTION

Lung is the site of rapid exchange of oxygen and carbon dioxide gases that is crucial for sustaining life. It has the largest epithelial surface of the body that is constantly exposed to air borne pollutants as well as pathogens. More than 99% of the internal surface area of the lung resides is in the alveolar compartment which is lined by type I and type II epithelial cells. Type II epithelial cells are granular pneumocytes consist of about 15% of the distal lung cells and occupy 5% of the alveolar surface [1]. Type II cells are considered to be the progenitor of type I cells [2]. Type II cells are smaller cuboidal cells that synthesize, secrete, and recycle surfactant components, transport ions participate in lung immune responses. In this mini-review, we summarize our results on isolation and purification of primary lung epithelial cells from mouse lungs, the changes in morphology and phenotype of cultured primary lung epithelial (PLE) cells and our data that suggests that the PLE cells may have an immunological function.

ISOLATION OF LUNG EPITHELIAL CELL

There are several methods of isolation of type I and type II cells in the literature [3]. We developed a method of isolation and culture of primary lung epithelial (PLE) cells that contained both type I and type II cells [4-6]. To isolate the lung epithelial cells mouse lungs were digested with dissociative enzymes (collagenase, protease, DNAse) to get a single cell suspension. Single cell suspension was a heterogeneous mixture of different kinds of cells including leukocyte, erythrocytes, endothelial cells, clara cells, goblet cells and muscle cells, etc. About 10-12 million cells could be derived from lungs of one mouse. PLE cells were purified from heterogeneous mixture by using a mouse epithelial cell enrichment kit containing immune-magnetic beads coated with monoclonal antibodies (anti-CD45, anti-TER119 and anti-CD31) to remove leukocytes, erythroid and endothelial cells respectively by negative selection as recommended by the manufacturer (Mouse Epithelial Cell Enrichment Kit, Stem Cell Technology, MA). Using this kit, approximately

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5×10^6 purified epithelial cells could be obtained per mouse lung. Relative proportions of macrophages and lymphocytes in freshly isolated lung epithelial cell preparations and after purification using the negative selection are shown in **Table 1**. Purified PLE cells in general had less that 2% contamination of macrophages and lymphocytes.

Table 1. Purification of PLE cells: Contaminating cells in PLE cell preparation before and after purification by negative selection using magnetic beads kit.

Contaminating cells (Markers)	PLE cells before negative selection (%)	PLE cells after purification by negative selection (%)
Macrophage (F4/80)	18.2 ± 0.4	2.9+0.4
T cells (CD3)	11.2 ± 0.7	2.0+0.4
B cells (B220)	8.0 + 0.3	<0.1%
NK cells (NK 1.1)	4.8 + 0.2	<0.5%

MORPHOLOGICAL AND PHENOTYPIC CHANGES IN CULTURED LUNG EPITHELIAL CELLS

Isolated PLE cells were cultured for 18-20 h in complete culture medium (RPMI 1640 with 10% FBS) after which, non-adherent cells, dead cells and cell debris was washed off. Left panel of **Figure 1** shows the appearance of PLE cells on day 1, 3 and 6 of the culture where an increase in size of cells is clearly seen. PLE cells were categorized as type I and type II on the basis of markers podoplanin (RT 140 for TI cells) and CD74 (for TII cells) present on the lung epithelial cells [7]. Freshly isolated PLE cells comprised both type I (about 20%) and type II cells (about 18%) as well as double negatives (DN cells about 55%) and double positive (DP cells, about 12%). When the purified PLE cells were put under culture condition percentage of type II cells under culture conditions progressively declined whereas the percentage of type I cells population increased. Percentage of TI cells (TI and DP cells) increased from about 31% to 90% from day 0 to day 4 whereas TII cell (TII and DP cells) decreased from 25% to 0.5%. Kinetics of change of proportion of type I and type II cells are shown in right panel of **Figure 1**. It is not clear whether the TI cells, whose proportion increased rapidly in culture, were derived from other cells (TII, DN or DP cells) or were derived from the cell division of other TI cells.

Proliferative activity of PLE cells was monitored by using live imaging time-lapse photography technique [6]. PLE cells were observed while kept in the incubation chamber of live cell imagine microscope (37°C, 5% CO_2 atmosphere) for 16 h and time lapse record examined for number of cells that divided within the view-field in that time period. Using this technique it was found that about 6% of the PLE cells divided between third and fourth day while about 11% of the PLE cells divided between 4^{th} to 5^{th} day in the culture which is predominantly type 1 cells. Our results indicated that the cell division activity started after second day in culture and peaked between days 4 to 5 and fell thereafter [6]. After fifth day the proliferative activity fell and by 7^{th} and 8^{th} day, cells became quiescent and devoid of any cell division activity.

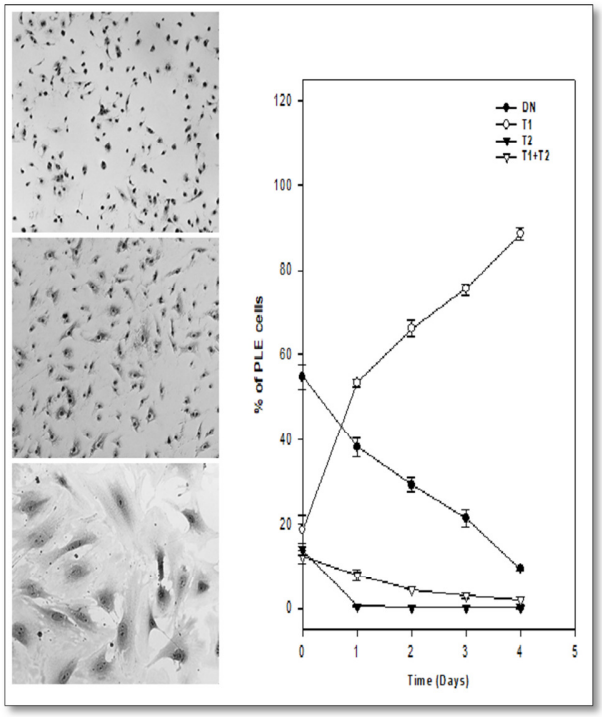

Figure 1. Morphological and phenotypic changes in cultures PLE cells. Left panel shows the morphology of PLE cells on day 1, 3 and 6 of the culture. In the right panel, the relative proportions of T1, T2, double positive (DP) and double negative (DN) cell populations have been shown. Freshly isolated PLE cells (day 0) and cultured PLE cells at different days (1, 2, 3 and 4) were double stained with anti-podoplanin mab (marker for Type I) and anti-CD74 mab (marker for type II epithelial cells) and analyzed on flow cytometer. DN (negative for podoplanin and CD74); T1-type I PLE cells; T2-type II PLE cells; T1+T2-type I (DP) and type II (positive for podoplanin and CD74) PLE cells. Percentage of DN, T1, T2 and DP cells population were calculated from FACS data. Each point represents mean ± SEM of 3 observations. Adapted from Kumari and Saxena [6].

EVIDENCE FOR A POSSIBLE ROLE OF PLE CELLS IN LUNG IMMUNITY

In view of constant on slaught of airborne pathogens and pollutants in lungs, keeping the crucial life-sustaining organ free of infections is a challenge. Since the cells that come in contact with the pathogens are predominantly lung epithelial cells that line the alveoli, it is possible that these cells may have some role to play in immune mechanisms aimed at killing the pathogens. Innate immune responses are in general inflammatory responses that tend to contain the infection and determine the quality of adaptive immune response to follow. Sensing of microbial antigens (pathogen associated molecular patterns or PAMPS) is believed to be through toll like receptors (TLRs) that send signals to start the secretion of inflammatory cytokines. Purified PLE cells were found to express TLR2 and TLR4 molecules and this expression increased significantly is BCG antigens were administered intra-tracheally (**Figure 2A**). Administration of pollutants like silica micro-fine particles suppressed the expression of TLR receptors (**Figure 2A**). Presence of TLR receptors on PLE cells suggest that these cells may participate in initial inflammatory reaction. However, for this purpose it was necessary to further show that PLE cells had the ability to secrete inflammatory cytokines in response to microbial antigens. Our results in **Figure 2B** show that cultured PLE cells secreted significant amounts of TNFα in response to sonicated BCG antigen as well as bacterial endotoxin LPS. Micro-fine silica particles too were effective in inducing TNFα secretion by PLE cells in culture. These results suggest that PLE cells may have a role in inducing inflammatory responses in response to microbial challenges and exposure to pollutants.

ANTIGEN PRESENTATION TO T-HELPER CELLS BY PLE CELLS

We have previously shown that like the professional antigen presenting cells (macrophages and dendritic cells) PLE cells too may have the ability of presenting peptide antigens as well as lipid antigens to T cells [4-6]. In this study, T helper cells were purified from the spleen of BCG immunized mice and co-cultured with peritoneal macrophages or PLE cells with a prior exposure to BCG. Representative results in **Figure 3** show that BCG sensitized T helper cells released IL2 as well as IFNγ when BCG antigen was presented by macrophages. These results are expected since macrophages are known to efficiently process and present peptide antigens to T helper cells [8]. However we found that the co-culture of sensitized T helper with BCG treated PLE cells also resulted in IL2 and IFNγ secretion by T helper cells, even though the cytokine release was marginally lower with PLE cells as APCs as compared to macrophages (**Figure 3**). Similarly, we could also demonstrate the presentation of lipid antigens by PLE cells [9]. Taken together, our results provide strong evidence for a possible role of PLE cells in both innate as well as adaptive immune responses to pathogen challenge in lungs.

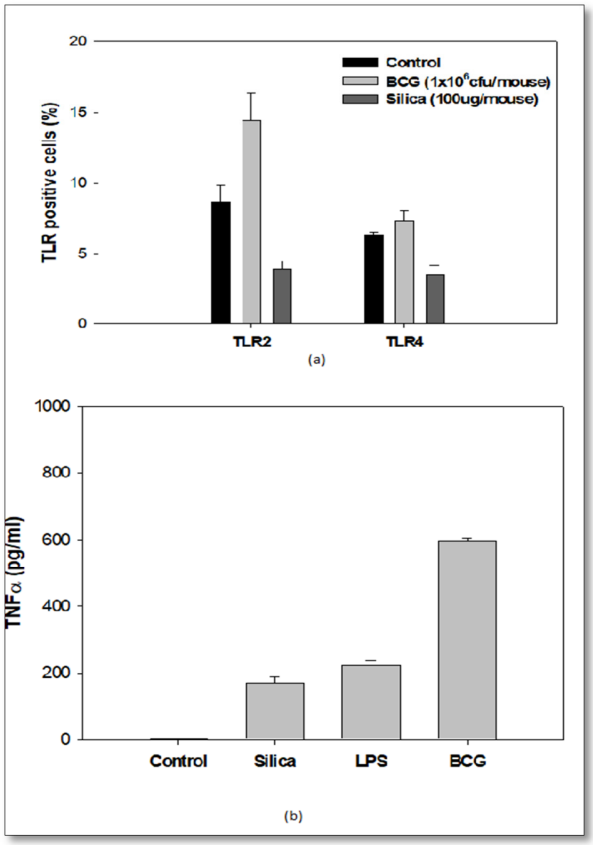

Figure 2. (Panel A) TLR expression on PLE cells and modulation by BCG and silica. BCG (1×10^6 cfu/mouse) or silica (100 µg/mouse) was intra-tracheally instilled into C57BL/6 mice. After 5 weeks, lung epithelial cells were isolated from control (PBS instilled), BCG infected and silica instilled mice. Cells were washed and stained with anti TLR2 and TLR4 antibodies. The stained cells were analyzed on flow cytometer. Results are expressed as Mean ± SEM of 3 observation. (Panel B) Effect of silica, LPS and BCG on TNFα production by PLE cells in culture. PLE cells were treated with silica (100 µg/ml), LPS (10 µg/ml) and BCG (MOI 100:1). After 24 h supernatants from treated and control cells were harvested and the levels of TNF alpha was measured by ELISA. Each point represents Mean ± SEM of values obtained from 3 replicate assay wells.
*$p<0.05$ for difference with control

Some reports in literature suggest that type II lung epithelial cells may present antigen. Debbabi et al. [10] isolated type II mouse lung epithelial cells which present Mycobacterium tuberculosis antigens to primed T cells but concluded that the epithelial cells are not able to prime naive T cells. Recently, Gereke et al. [11] have shown that type II lung epithelial cells can prime and activate naive T cells to alveolar self-antigens. The present short review of our own work suggests a possible role of type I PLE cells also in the development of adaptive immunity.

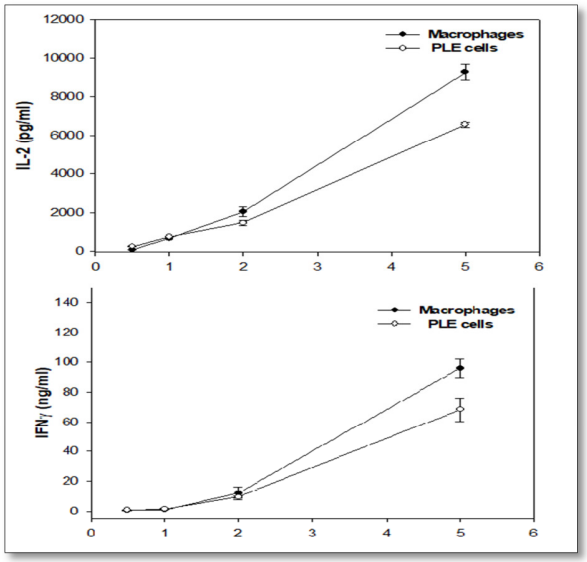

Figure 3. T-helper cytokine response to BCG antigen while using macrophages (closed circles) or PLE (open circles) as APCs. Different numbers (0.5×10^5, 1×10^5, 2×10^5 and 5×10^5) of PLE cells and peritoneal macrophages were cultured with BCG sonicate (MOI 100:1) for 24 h. Both APCs were fixed with glutaraldehyde and quenched with L-lysine as described in Kumari and Saxena [4]. Purified CD4+Th cells from BCG infected mice were added to the wells at T cell/APC ratio of 1.5. After 24 h of co-culture, the amount of IL-2 (panel A) and IFNγ after 48 h (panel B) secreted by T-helper cells in culture supernatants was quantified by ELISA. Each point represents Mean ± SEM of values obtained from 3 replicate assay wells #$p<0.05$ difference between epithelial cells and macrophages. Adapted from Kumari and Saxena [4].

Since type I PLE cells line the alveolar space where gaseous exchanges take place, these cells become the very first layer of cells that separate the airborne pathogens and pollutants from the internal sterile milieu of the body. It would therefore be quite appropriate if specialized mechanisms have evolved at this surface to help prevent the penetration of infectious agents. Our results point out that the PLE cells indeed have some ability to contribute to the immune mechanisms that operate in alveoli. Further work is needed to understand the nature of these mechanisms and the collaboration of PLEs with alveolar macrophages to provide a formidable layer of defence against pathogens in the lungs.

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