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Research paper

# Collagenase digestion down-regulates the density of CD27 on lymphocytes

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ABSTRACT

Collagenases are widely used for tissue digestion in experiments due to their potent hydrolysis of connective tissue. CD27, also known as tumor necrosis factor receptor superfamily 7 (TNFRSF7), is limited to be expressed on the cells of the lymphoid lineage. In our preliminary research, we found that CD27 on NK cells was almost disappeared with the digestion of type I collagenase for 90 min. This phenomenon suggests that the process of tissue digestion may affect the density of CD27 on cells. In order to verify this, the lungs of mice were digested with types I and IV collagenase or grinded, respectively. The percentage of $CD27^+$ cells and the density of CD27 on cells were assayed by flow cytometry. The data presented that the percentage of $CD27^+$ cells and the density of CD27 on lymphocytes gradually decreased with the time of digestion with type I or IV collagenase. We also detected that the density of CD11b on NK cells was not affected by collagenase digestion. Collectively, the findings of the present study suggest that the collagenase digestion has a selective effect on the density of molecules on cells.



## 1. Introduction

Collagenase are enzymes produced from *Clostridium histolyticum* (Seifter et al., 1959). The original use of collagenase to digest lung could be date to 1984 when P. G. HOLT et al. reported a method for the quantitative extraction of pulmonary interstitial cells (Timonen and Stenius-Aarniala, 1985). And before that, the lower respiratory tract was restricted to experiments involving cell acquisition by bronchoalveolar lavage which has a deficient that the cell types within bronchoalveolar lavage fluid (BALF) could not necessarily reflect the content of the lung interstitial cell pools (Mauderly, 1977). The procedure of collagenase digestion of whole lung involves the incubation of perfused lung in a mixture of collagenase, DNase and fetal calf serum, followed by recovery of cells on a discontinuous percoll gradient. This method could obtain live cells from the lung in the greatest extent. Many laboratories have adopted this approach to digest lungs of various animals (Schultheis et al., 1994; DeLorme et al., 2002; Bassetti et al., 2010; Wilson et al., 2013). However, the defect of the collagenase digestion is not fully clear.

CD27 is a 45 kD trans-membrane protein which belongs to tumor necrosis factor receptor superfamily (TNFRSF) and is exclusively expressed on lymphocytes including nature killer (NK) cell, T and B cell population (Lens et al., 1998). Murine NK cells can be divided into four subsets according to the surface density of CD11b and CD27 as the most immature $CD27^{low}CD11b^{low}$, immature $CD27^{high}CD11b^{low}$, mature $CD27^{high}CD11b^{high}$ and most mature $CD27^{low}CD11b^{high}$ subsets (Silva et al., 2008; Chiossone et al., 2009). CD27 also plays a critical role in T cell maturation, expansion, and memory and contributes to the differentiation of $CD8^+$ T cells to effector cytotoxic T lymphocytes (CTLs) when engaged by its ligand CD70 (Brown et al., 1995; Hendriks et al., 2000; Jang et al., 2013). Besides, CD27 represents an important marker for memory B cells, and the CD27 signaling promotes the differentiation of

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memory B cells into plasma cells (Agematsu et al., 1997; Berkowska et al., 2011). Thus, the density of CD27 on cell surface influences the lymphocytes' maturation, activation, proliferation, differentiation and function.

In our preliminary experiment, we found very low density of CD27 on NK cells after digesting the lungs of mice with type I collagenase for 90 min. This phenomenon suggests that collagenase affects the density of CD27 on cells. To confirm this hypothesis, we compared the density of CD27 on cells obtained from the lungs of mice by grinding and traditional types I and IV collagenase digestion respectively.

## 2. Materials and methods

### 2.1. Mice

Specific pathogen-free female BALB/c, six-wk-old, were obtained from Centers for Disease Control (CDC), Wuhan (Hubei, China). All methods and protocols involving mice were approved by Animal Care and Use Committee of Tongji Medical College, HUST (China).

### 2.2. Lung digestion and recovery of live cells

The mice lungs perfused with saline containing 0.5 mM EDTA were immediately excised and cut into pieces to incubate in 3 ml of complete RPMI 1640 (Hyclone, Thermo Fisher Scientific), supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 mg/ml streptomycin, DNase I (0.1 mg/ml) and/or type I collagenase (0.5 mg/ml) or type IV collagenase (0.5 mg/ ml) (all from Sigma Aldrich, Saint Louis, USA) for 30, 60 or 90 min in a shaking 37 °C air bath. The mixture was then filtrated through a 200-mesh stainless-steel gauze supplemented with complete RPMI 1640 and centrifuged at 300 ×*g* for 10 min. The cell pellet was resuspended in 1 ml PBS and added on 2 ml 33% Percoll (GE Healthcare, Amersham, UK) slowly and centrifuged at 400 ×*g* for 30 min at room temperature. The cells at the bottom of centrifuge tube were harvested and then washed twice with PBS and resuspended in PBS.

### 2.3. Lung grinding

The lung pieces were ground with a stirring rod accompanying complete RPMI 1640 adding on a 200-mesh stainless-steel gauze which settled on a 50-ml polyethylene centrifuge tube, and the cells were filtrated and centrifuged at 300 ×*g* for 10 min and resuspended in PBS (Fig. 1).

### 2.4. Flow cytometry analysis

Cells isolated from lungs were incubated for 30 min with optimal concentration of PE/Cy7-conjugated anti-CD3 mAb, PerCP/Cy5.5-conjugated anti-CD11b mAb, APC-conjugated anti-CD27 mAb, PE-conjugated anti-B220 mAb (all from Biolegend) and FITC-conjugated CD49b mAb (eBioscience). Cells were washed twice and then detected by flow cytometry (FACS Calibur; BD Biosystems, San Jose, CA) and analyzed using FlowJo 7.6 software (Acresso).

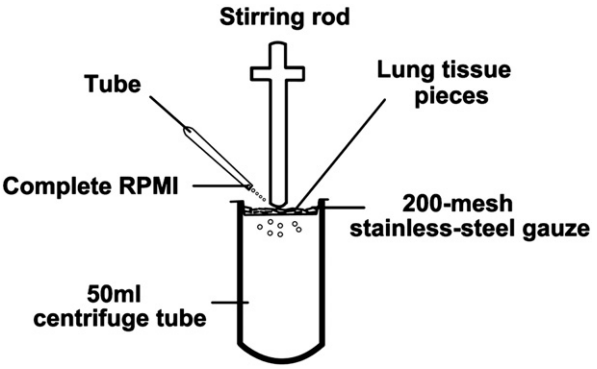


**Fig. 1.** The scheme of lungs grinding. A 200-mesh stainless-steel gauze was settled on a 50 ml polyethylene centrifuge tube in which the cap has been unscrewed. The lung pieces were ground on the gauze using a stirring rod, and the cells were filtrated through the gauze into the tube by adding complete RPMI 1640 simultaneously.

### 2.5. Statistical analysis

Results are shown as the mean + SEM. Statistical significance of differences was assessed by two-tailed Student's *t* test. The software package GraphPad Prism 5 (GraphPad Software, San Diego, CA) was used for data analysis. Values of $p < 0.05$ were considered significant.

## 3. Results and discussion

The preliminary experiment suggests that the collagenase digestion may have an impact on the density of CD27 on cells and as this molecule was limited to cells of the lymphoid lineage, all the lymphocytes obtained from lungs were gated according to forward scatter (FSC) and side scatter (SSC) (Fig. 2A). NK cells ($CD3\text{-}CD49b^+$), T cells ($CD3^+$) and B cells ($B220^+$) within lymphocytes were gated for the following analysis, respectively (Fig. 2A). The percentages of $CD27^+$ lymphocytes, $CD27^+$ NK cells, $CD27^+$ T cells and $CD27^+$ B cells had a time-dependent decrement with the treatment of collagenase I compared with grinding (Fig. 2B). There was no obvious difference of the percentages of $CD27^+$ lymphocytes, $CD27^+$ T cells and $CD27^+$ B cells between grinding and collagenase I digestion for 30 min, however the percentages of $CD27^+$ lymphocytes, $CD27^+$ T cells and $CD27^+$ B cells were decreased significantly with collagenase I digestion for 60 and 90 min (Fig. 2B). The percentages of $CD27^+$ NK cells in all of collagenase I digestion for 30, 60 and 90 min groups were decreased significantly compared with the grinding group (Fig. 2B). The density of CD27 presented as mean fluorescence intensity (MFI) on lymphocytes, NK cells, T cells and B cells also had a time-dependent decrease with the treatment of collagenase I compared with grinding (Fig. 2C). DNase I digestion and percoll treatment had no significant impact on the percentage of $CD27^+$ cells and the density of CD27 on cells.

To investigate whether the effect of digestion on the density of CD27 was exclusively limited to type I collagenase, we digested the lungs with type IV collagenase, another type of collagenase, which is also commonly used for lung digestion in animal experiments. The result showed that the percentage of $CD27^+$ lymphocytes was gradually decreased with the time of