The Effect Collagen to Granuloma Structure and Immune Response on Granuloma Tuberculosis In Vitro Models

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Abstract: Mycobacterium tuberculosis is a bacterium causes pulmonary tuberculosis, acid-resistant bacteria, intracellular life and cause of death than other infectious diseases. The principle of immune response by the formation of granulomas that prevent bacteria spread to other cells. TNF-α plays an important role in the body's defense against intracellular bacterial infections and the invitro model maintaining granulomas and the role of collagen in maintaining granulomas that prevent infection from becoming active. The purpose of this study is to analyze the number of lymphocyte, monocytes/macrophages and levels of TNF-α between collagen and without collagen. This study used healthy PBMC cells in mycobacterium tuberculosis bacterial infection with MOI 1: 0.1 then divided into 2 groups the addition collagen and without collagen observation in day 1, day 3, day 5, and day 7. HE stained was performed to calculate lymphocyte, monocyte/ macrophage and supernatant cells used to check TNF-α levels by ELISA method. The results showed no effect of the addition of collagen to the number of lymphocytes, monocytes/macrophages and TNF-α levels. Levels of TNF-α highest on day 3 and decresed on day 7.

1 INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacterium Mycobacterium tuberculosis has caused more deaths during the last 200 years compared to other infectious diseases (Paulson, 2013). World Health Organization (WHO) data by 2015, an estimated 10.4 million new TB cases worldwide. Six countries accounted for 60% of new TB cases, including India, Indonesia, China, Nigeria, Pakistan and South Africa (WHO, 2016).

Tuberculosis disease in addition to causing active infection can also cause latent infections that are asymptomatic conditions making it difficult for treatment because it does not cause symptoms. In the host body infected with Mycobacterium tuberculosis mostly develops into a latent infection in comparison with active infection at the host's primary defense against Mycobacterium tuberculosis infection with granuloma formation, ie the formation of organized cell aggregates. (Fitzgerald et al., 2014).

Mycobacteria belong to the host by means of aerosol will then be in the alveolar macrophages by internalization by way of Mycobacterium tuberculosis bacteria secrete virulence factors namely ESAT-6 (Early Secreted Antigenic Target of 6 kDa), which in the secretion of through the system the secretion of Mycobacterial type VII (ESX-1). ESX-1 mediated translocation m.tuberculosis from the macrophage cytoplasm into a phagolysosome is the virulence of pathogenic Mycobacteria (Parasa et al., 2014).

Mycobacterium tuberculosis bacteria are internalized by macrophages releasing Interleukin 8 (IL-8) which is a strong chemoatractant for T lymphocytes because it produces a bacterial antigen so as to recruit T cells. T lymphocytes then become active and produce gamma interferon (IFN-γ) which serves to activate other macrophages , increasing the production of Tumor Necrosis Factor Alpha (TNF-α) and activating the metabolism of oxidative macrophages and antimicrobial activity. TNF-α plays a very important role in the development of granulomas for cellular organization and maintenance of granulomas, TNF-α mediates acute phase responses that give rise to systemic symptoms,

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and is necessary for the development and the strength of granulomas that play an important role in the accumulation and differentiation of macrophages into epithelioid cells on mature granulomas (Birkness et al., 2007 and Fitzgerald et al., 2014).

Granulomas are not always awake, bacteria Mycobacterium tuberculosis can be reactivated. During reactivation, granulomas become catalase and then necrotic, and infected macrophages secrete MMP-1, excessive secretion leads to collagen degradation and tissue damage, causing M. tuberculosis to enter the respiratory tract and causing active infection. (Salgame, 2011)

The damage to collagen is an early onset of immunopathology in tuberculosis, causing necrosis and building an immune response, revealing the role of extracellular matrix in regulating host and pathogen interactions (Al Shammari et al., 2015)

Type I collagen is a fibril structure in the lung and is highly resistant to enzymatic degradation. In addition to biomechanical properties, type I collagen has an important role in cell survival, adhesion, proliferation, and migration (Brilha et al., 2017).

This study reports on the effect of collagen administration on the structure of granuloma and the secretion of Tumor Necrosis Factor (TNF) -α in granuloma tuberculosis in vitro models.

2 MATERIALS AND METHODES

2.1 RPMI

Roswell Park Memorial Institute (RPMI) 1640. RPMI 1640 media is a medium used for cell and tissue culture, usually used for the growth of human lymphoid cells. RPMI 1640 uses the bicarbonate buffer system so that it enables the growth of several types of cells, especially T lymphocytes, hybridomas.

2.2 PBMCs

Peripheral Blood Mononuclear Cells = PBMCs are cells made from human blood which are then processed for the PBMC cell capture. The concentrations of PBMC used in this study was 10^6 in each well.

2.3 Mycobacterium Tuberculosis

This study used bacterial isolates Mycobacterium tuberculosis strain H37Rv with concentration 10^5 in each well.

2.4 Extracellular Matrix

The extracellular matrix used collagen. The collagen used has a number Cat # 04902 as a solution. In this study, 950 µL of collagen was added with 50 µL PBS 10 × and add 10uL NaOH 1N, then in PH check, with neutral PH result.

3 RESULTS

3.1 Direct Granuloma Observation

The method used for direct observation is performed directly under an inverted microscope using specimens of living cell cultures in the plate / well.

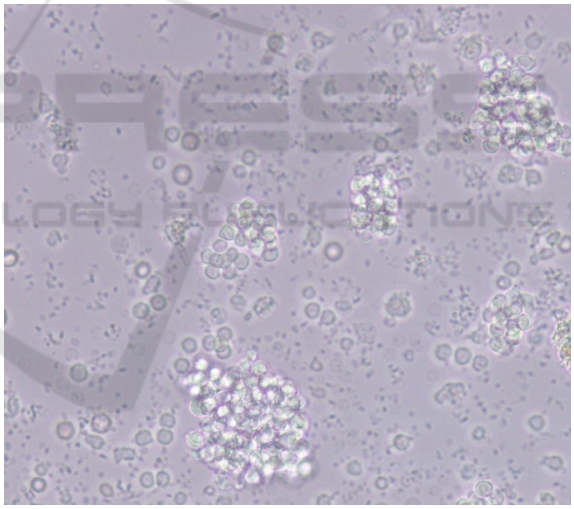

Figure 1: Direct observation with infection (400x magnification)

The figure 2 seen there is aggregate it indicates a response to infection.

3.2 Calculation Count of Cells on the Structure Granuloma

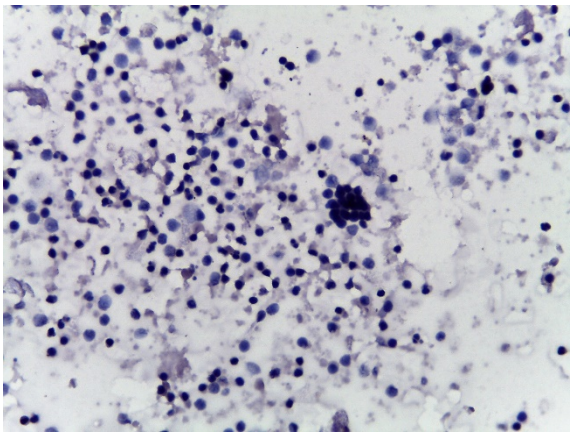

Figure 2: HE staining (400x magnification)

Figure 2 is the result of HE staining, the measurement of lymphocyte cell distance and monocyte / macrophage cells can be identified and calculated on the granuloma structure.

Table 1. Average amount of cells constituent of granuloma structure

Treatment	Cells	Day			
		1	3	5	7
With Collagen	lymphocyte	14.5	11.33	5.17	2
	monocyte / macrophage	1.33	1.16	0.83	0.5
Without Collagen	lymphocyte	10.5	9.83	3.83	2.333
	monocyte / macrophage	1.33	1	0.67	0.33

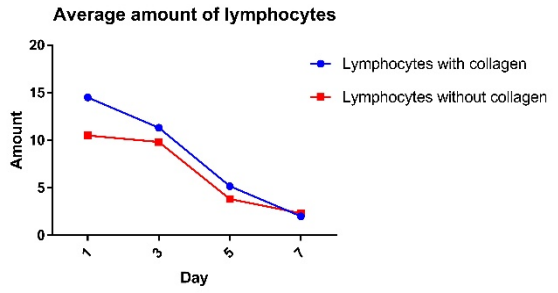

Figure 3: Average amount of lymphocytes

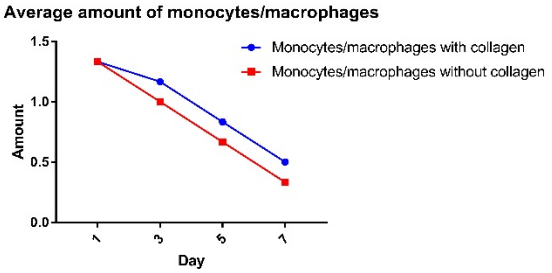

Figure 4:Average amount of monocytes/macrophages

Average number of cells of lymphocytes and monocytes/macrophages shown in Figure 3 and Figure 4. With paired t test P value of lymphocyte cell count = 0.1662 and monocyte cell number the value P = 0057 so there is no effect of collagen on the amount lymphocyte and monocytes/macrophages.

3.3 Examination the Levels of TNF-α

Table 2. Average levels of TNF-α

Treatment	Day (pg/ml)			
	1	3	5	7
With Collagen	321.051	808.725	299.990	249.786
Without Collagen	340.997	496.227	272.972	245.512

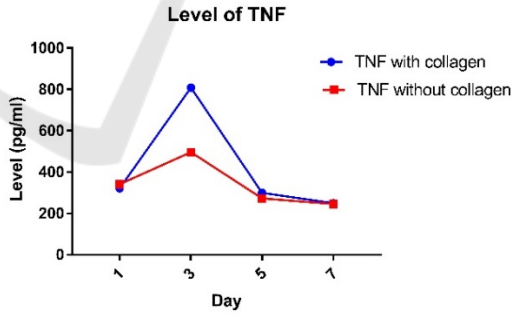

Figure 5: average levels of TNF-α based on variations day With Paired t test P-value = 0.3744 or P > 0.05 so there is no effect of collagen on the TNF-α.

3.4 Discussion

The formation of a granuloma is a dynamic process. Formation of granuloma can be divided into three phases, first phase is the stage where the innate granuloma formed from macrophages and neutrophils. The second phase immune granuloma

formed after the emergence of specific antigen of T cells. The third phase chronic granuloma comes from the difference in the morphology and the change of the structure of the granuloma. After the M. Tb root is infected with Alveolar Macrophages (MA) then it goes earlier against the inflammatory response. In the meantime, it strengthens the immune response of the host then the recruitment of innate immune cells against the new target M. Tb and contribute to spreading M.Tb. The mycobacterium species is inhibited by the fusion process fagolysosome. It is associated with virulence, the strain of relativity M.Tb inhibits fusion. Infected macrophages produce a number of pro-inflammatory cytokines and chemoatractant cytokines TNF-α, IL-6 and IL-8, which facilitate the recruitment of macrophages and granulocytes into new infections and lead to the formation of congenital granulomas (Shaler et al., 2013).

The chronic Granuloma phase causes significant changes in morphology and granuloma function. In infected individuals a spectrum of granuloma structures, the classification of either bacterial or non-bacterial lesions and fibrotic necrotic granulomas, suggests that granuloma evolution is a highly dynamic process (Shaler et al., 2013).

The pathologic infection of tuberculosis in humans is an organized aggregate granuloma that is organized from immune cells consisting of macrophages, lymphocytes and immune cells present in the host (Cadena A.M, et al, 2017). Formation of granuloma phase of immune granuloma will produce continuous chemokine by APC in infected lung and efficiently recruit T cells. Then, T cells will surround and close the infected macrophages by M.Tb bacteria. T cell activation serves as bactericide and limits bacterial mobility thus it prevents the spread of bacteria to other cells. The arrival of T cells and the formation of immune granulomas are associated with the growth of stable bacteria (Mogues et al., 2001).

Monocyte / macrophage cells will clump in response to Mtb infection and form a structure such as granuloma and initiate granuloma formation. It is defined as a grouping of monocytes / macrophages during inflammation, an initial occurrence during mycobacterial infection (Parasa et al, 2014).

Macrophages will secrete IL-8 as a strong chemoatractant for T lymphocytes that will surround the granuloma structure. T lymphocytes then secrete IFN-ɣ to activate additional macrophages. Macrophages will produce TNF-α and play an important role in the accumulation of macrophages and other cells (Fitzgerald et al., 2014).

Tumor Necrosis Factor Alpha (TNF-α) is a cytokine that emerges since early inflammation plays an important role in the mechanism of the innate immune response. TNF-α is an autocrine cytokine produced by macrophages, dendritic cells, lymphocytes, neutrophils, mast cells, and endothelial cells and performs functions such as chemotaxis with the formation of granulomas (Sasindran and Torrelles, 2011).

4 CONCLUSIONS

The invitro is a risky method, since the contamination is likely to occur. Therefore, it is suggested to absolutely ensure the sterile condition before conducting the isolation, the tools, materials, specimens.

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