

## Original Research

### Phagocytic responses of peritoneal macrophage white rats to neutral red against plastic

Winnie Yohana,

Oral Biology Department of Faculty of Dentistry, Padjadjaran University, Indonesia

#### ABSTRACT:

**Introduction:** The process of digesting germs or foreign particles is carried out by macrophages. There are various macrophages according to location of the macrophages, such as; alveolar macrophages (dust cell), liver macrophages (kupffer cell), skin macrophages (Langerhans cell), bone macrophages (osteoclast cell), peritoneal macrophages and others. Macrophages are specialized phagocytes that play an important role in against foreign invasion. The purpose of this study is to determine whether peritoneal macrophages that have been stained by neutral red, are they still able to do phagocytosis again. **Method:** This study was experimental laboratory research which was performed in two group, the first group was control group. The second group was those given neutral red injection, and each rat put 4 plastics into his abdomen. The rats were sacrificed after treatment, and made histological slide with Giemsa staining for peritoneal macrophages observation. The data was analyzed by using Cochran analyzed. **Result and Discussion:** The results show that were six histological slide, and each slide is examined on six views. Then there were 48 peritoneal macrophage descriptions were observed. The observation turned out to be 95.83% there were peritoneal macrophages that have bubbles and attached to the plastic, whereas in the rat control no bubbles were found. **Conclusion:** This study suggested that there was a relationship between twice phagocytic with peritoneal macrophages which contain neutral red, then attached to the plastic.

**Key words:** peritoneal macrophages, neutral red, phagocytosis, plastic.

Received: 26 October, 2019

Revised: 21 November, 2019

Accepted: 23 November, 2019

**Corresponding author:** Dr. Winnie Yohana, Oral Biology Department of Faculty of Dentistry, Padjadjaran University, Indonesia

**This article may be cited as:** Yohana W. Phagocytic responses of peritoneal macrophage white rats to neutral red against plastic. *J Adv Med Dent Scie Res* 2020;8(1):207-210.

#### INTRODUCTION

Macrophages are specialized phagocytes that play an important role in clearance of effete host cells and molecules, as well as defense against foreign invasion, including infection and biomaterial implantation.<sup>1,2</sup> Macrophages are dominant infiltrating cells that respond rapidly to biomaterial implantation in soft and hard tissues.<sup>3</sup> Macrophages are phagocytic cells that have single nuclei. In human body, and in vertebrates generally, the most effective phagocytic are two kind types of cells that specialize in phagocytosis, namely mononuclear phagocytes and polymorphonuclear leucocytes. Cells that are classified as mononuclear phagocytes are monocytes, macrophages, and polymorphonuclear leucocytes are neutrophils, eosinophils and basophils.<sup>2,4</sup>

Macrophage cells are important in the body's defense process against microorganisms and tumor cells, wound healing, and lipid metabolism.<sup>1,2,5</sup> Macrophages have the property that can phagocytosis of foreign objects,<sup>3</sup> such as neutral red. The function of phagocytosis can be demonstrated in vitro by showing other macrophage properties, macrophages can attach to rough surfaces such as cloth, glass fiber, plastic, and glass. Transparent plastic, thin, translucent, is another alternative that can be used in a research, then can be observed with a light microscope. An advantage of plastic is readily available in affordable price. The purpose of the study is to determine whether peritoneal macrophages that have been stained by neutral red (macrophages have phagocytosis process) whether they can still do phagocytosis again.

**METHOD**

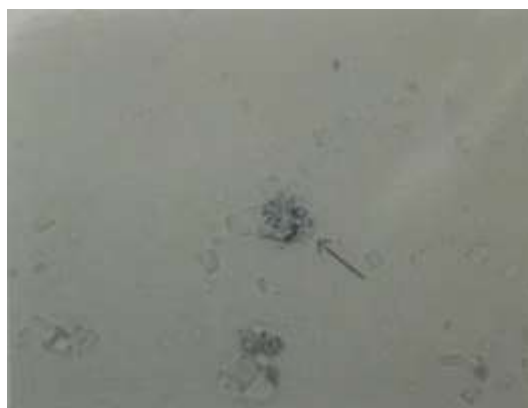
This was an experimental laboratory research which was performed in two groups of rats. The first group was the control group. The second group were those injected by neutral red. As the object of this study were female white rats weighing around 400gram, then the subjects of the study were peritoneal macrophages. A variable is neutral red on macrophages that can attach to the plastic, while the dependent variable is twice phagocytosis. An indicator of twice phagocytosis is the presence of dark blue bubbles. Rat was anesthetized with ethyl chlorine, after being anesthetized then injected by neutral red, then the rat is sacrificed. Opened her abdominal cavity, then put 4 pieces of transparent plastic size 3x0.75 cm, then the abdomen is sewn again, and the rat is left for 1-hour. After 1-hour the stitches were opened, the plastic was removed carefully, then the plastic was washed with physiological NaCl, then fixed with absolute alcohol. Plastic was immediately stained with Giemsa, the excess Giemsa solution was washed again with physiological NaCl and made histological slide. Then made a same procedure for a group control. The data obtained were coded, arranged in tabular form. The data of peritoneal macrophages were tested using Q-Cochran association test.

**RESULTS**

Histological slide can be observed under a light microscope with 100x magnification. The slide were reviewed for each part by observing 6 visual field view. Observation with 400x magnification aims to see more clearly peritoneal macrophages. The research variable was neutral red in macrophages that can attach to the plastic, while the dependent variable was twice phagocytosis. An indicator of twice phagocytosis was a presence of dark blue bubbles and attach to the plastic. To measure an indicator twice phagocytosis used nominal measurements as a symbol. The number 1 is used for peritoneal macrophages that have a dark blue bubbles attached to the plastic. The number 0 to indicate the absence of dark blue bubbles and attached to the plastic. Observation by microscopic examination with magnification 100x, and 400x. The observation frequency was carried out 6x, where each time the observation consisted of 8 slides (n = 8). Furthermore, the observations can be seen in table 1.

Research results can be observed under a light microscope with the following circumstances. In general, all histological slides have peritoneal macrophages that were irregular oval in shape with rough edges. Peritoneal macrophages contain neutral red attached to the plastic presented in Figure 1. From all studies there were 23 macrophages that were clearly visible (95.83%), while a group control no have neutral red in peritoneal macrophages (Figure 2). The data obtained were coded, arranged in tabular form, to realize there is a relationship between twice phagocytosis with neutral red contained in macrophages attached to the plastic, the Q-Cochran association test was used.

**Figure 1:** Peritoneal macrophages have bubble contain neutral red (arrow)  
It can be observed under a light microscope with 100x magnification.



**Figure 2:** In group control: peritoneal macrophages have no bubble (arrow)  
It can be observed under a light microscope with 100x magnification



**Table 1:** Peritoneal macrophages that phagocytic neutral red and adhere to the plastic

Description	Observation						Total
	1	2	3	4	5	6	
There are bubbles on the plastic	4	4	3	4	4	4	23
There are not bubbles on the plastic	0	0	1	0	0	0	1
Total	4	4	4	4	4	4	24
% bubbles on the plastic	100	100	75	100	100	100	

## DISCUSSION

Macrophages are specialized phagocytes that play an important role in clearance of effete host cells and molecules, as well as defense against foreign invasion, and biomaterial implantation.<sup>1</sup> In general, all histological slides have peritoneal macrophages that were irregular oval in shape with rough edges. In this study there were peritoneal macrophages contain neutral red attached to the plastic. From all studies there were 23 macrophages that were clearly visible (95.83%), this was consistent with Xia's research, that macrophages are dominant infiltrating cells that respond rapidly to biomaterial implantation in soft and hard tissues.<sup>3</sup> Macrophages are found to actively respond to almost all biomaterial implants *in vivo*, including ceramic, metal, polymer, protein such as collagen.<sup>3</sup> As macrophages are members of the host immune system, their responses to biomaterials have attracted wide concern. Macrophages activities are closely related to defense against microbe infection, activating inflammation and reacting against foreign body or particle invasion.

Major function of macrophages is to mediate host immune and inflammatory responses against foreign objects. For this reason, a clear understanding of the complex interaction between macrophages and biomaterial is crucial for improvement of material employed in the construction of biomedical devices.<sup>3</sup>

After recognition of foreign materials, macrophages need to bind a material before further action can be taken. Macrophages bind to foreign material via integrin-mediated adhesive interaction.<sup>6</sup> Normal macrophages naturally bind to foreign materials and mediate the biomaterial foreign body respon.<sup>7</sup>

The first step in the macrophage response to foreign invasion is to recognize self and non-self. Self recognition is the process by which in the immune system of an organism distinguishes between the body's own chemical, cells, tissues and those of foreign agents.<sup>8</sup> This was consistent with this study, that peritoneal macrophages have been stained by neutral red.

After phagocytosis, macrophages membrane remodeling leads to the complete enveloping of the particles and their release in the cytoplasm in novel membrane bound organelles the phagosomes, this was consistent with this study.<sup>9</sup>

Data are arranged in table 1, then data from peritoneal macrophages that have phagocytosis neutral red and attached to the plastic, data were analyzed using the Q-Cochran association test. After analyzing it turns out that the value of Q count = 3, and Q table = 0.58. Q count > Q table, that means there was a relationship between twice phagocytosis with neutral red in macrophages, which are attached to the plastic. The process of digesting germs or foreign bodies by certain cells in inflammatory reactions is called phagocytosis. Aschoff proposes a grouping of cells in the reticuloendothelial system, so that members of macrophage cells become more extensive, such as

histiocytes, alveolar macrophages (Dust cells), Pleural macrophages, liver macrophages (kuppfer cells), macrophages in the brain (microgloa), skin macrophages (Langerhans cells), macrophages in bones (osteoclasts), and peritoneal macrophages.<sup>2,5</sup> In 1971 a classification was carried out based on the similarity of its origins, namely from hematopoietic tissue and morphological properties as well as cell functions that have the ability to phagocytic foreign bodies or damaged tissue,<sup>10</sup> the known material is then digested in its cells by endocytosis in bubble shape.

Phagocytosis is a process that consists of 3 phases, namely: adhesion phase, ingestion phase, and digestion phase.<sup>11</sup>

This phagocytic process can occur, both in small molecules and large molecules, for example against neutral red dyes. Phagocytosis is mostly carried out by macrophages, for example peritoneal macrophages that are included in macrophage wandering with a size of 10-30 microns. Mononuclear phagocytes have the ability to move in tissues take place randomly or directed to a chemical stimulus. This movement is thought to be due to the secretion of proteolytic enzymes that will blaze their ways.

Macrophages that are active usually always experience changes in shape and structure, this can be seen by the rapid change of shape to flat on the glass / plastic with a jagged boundary. Also in the cytoplasm contains a lot of lysosomes, and more phagocytic. Activation of macrophages can be done with mediators produced by lymphocytes, both stimulated by antigens and endotoxins. Furthermore, he processed it, and gave the results of his work to T lymphocytes or B lymphocytes for subsequent immunological reactions.<sup>3,12</sup> Thus macrophages play an important role in initiating and regulating the immune response. Macrophages play a role in the body's defense against microorganisms and tumor cells, helping in the supply of granulocytes and erythrocytes through the production of colony stimulating factors, besides that macrophages also help in lipid metabolism.<sup>3</sup>

## CONCLUSION

This study suggested that there was a relationship between twice phagocytosis with peritoneal macrophage which contain neutral red, then attached to the plastic.

## REFERENCES

1. Gordon S. Pattern recognition receptors: doubling up for the innate immune response cell. 2002;111(7):927-30
2. Furt V R, Cohn A Z, Hirsch G J, Humphrey H J, Spector G W, Langevoort L H. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. Research gate. 2015:845-52
3. Xia Z, Triffitt T J. A review on macrophage responses to biomaterials. Biomed. Mater. 2006;R1-R9
4. Gartner P L, Hiatt L J. Color textbook of histology. Maryland: Saunders. 2<sup>nd</sup> ed. 2001:120-2

5. Yona S, Gordon S. From the reticuloendothelial to mononuclear phagocyte system-the unaccounted years. 2015;6:328
6. Phillips, Jeffrey M, Kao J W. Macrophage adhesion on gelatin based interpenetrating networks grafted RGD. Tissue Engineering. 2005:964-73
7. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical protein linking innate and adaptive immunity. Nat. Immunol. 2001:677-80
8. Chaplin D V. Overview of the immune response. J. Allergy Clin. Immunol. 2010;125(2 Suppl2):S3-23
9. Jutras I, Desjardins M. Phagocytosis: at the crossroads of innate and adaptive immunity. Annu. Rev. Cell. Dev. Biol. 2005:511-27
10. Rieger A M, Schroeder T. Hematopoiesis. Cold Spring Harbor Perspect Biol. 2012;4(12):a008250
11. Rosales C, Querol U E, Phagocytosis: A fundamental process in immunity. Biomed. Res. Int. 2017: PMC 5485277. PMID: 26891037
12. Mosser M D, Edwards P J. Exploring the full spectrum of macrophage activation. Nat.Rev Immunol. 2008; 8(12):958-69