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A Flow Cytometric Investigation of Neutrophiles Percentages in the Rabbit Peripheral Blood during an Induced Infection

GERALD MUÇA[1], ENKELEDA OZUNI, ALBANA MUNGA, XHELIL KOLECI, NAJADA MALAJ, LILIANA TAIRLLARI, ERINDA DAUTAJ, BELINDA DHAMI, PËLLUMB ZALLA, NERTILA DINI, DORIANA BEQIRAJ, MAJLIND SULÇE

Faculty of Veterinary Medicine, Agricultural University of Tirana, Rruga Paisi Vodica, 1029, Kodër Kamëz

Abstract

In normal conditions Neutrophils are listed as the first leukocytes population that reaches the infection site, moving toward the site through chemotaxis due to their response to chemical signals produced. Neutrophils circulate in the peripheral blood representing the major percentage of white blood cells ranging from 45 to 80% of all populations. This percentage can be different in different species. Flow cytometry represents a relatively new technique in veterinary medicine that has many functions, especially in identifying different cell populations using specific antibodies. Our aim was to observe the Neutrophilic movement in the peripheral blood when determined quantity of E. Coli Lypopolysaharides are administered to a specific site in rabbits. Thus, five healthy rabbit individuals were artificially infected, and peripheral blood was collected in different moments: prior to the infection and in different specific moments after the infection. Percentage of Neutrophils was detected in each collection moment in order to evaluate their presence in the peripheral blood. Results showed that number of Neutrophils decreases significantly during the first thirty minutes considered as T1, compared to T0. Their number increases significantly after the first two hours. Based on these results we hypothesized that Neutrophils reached the site of infection and after, a general sensitization occurs due to the infective status. However further studies with a higher caseload are warranted to better define the association between differences in Neutrophils percentage in the peripheral blood and the infectious dose.

Key words: Neutrophils, rabbit, flow cytometry, infection, blood

INTRODUCTION

New infection status inside the organism may induce a Neutrophilic response in this regard in terms of their concentration in the peripheral blood. Their movement toward the site of infection is due to their response to the infection site through chemotaxis (De Oliveira et al., 2016, Petri and Sanz 2018, Metzemaekers et al., 2020, Zhang et al., 2024). This mechanism is well known even if recently, some abnormalities during sepsis have been showed since, the migration abilities and antimicrobial functions of neutrophils are impaired, resulting in a dysregulated immune response (Zhou and Sun 2022). The movement of Neutrophils during the presence of an acute infectious status can be observed by different techniques. It is important to emphasize the fact that the

[1] Corresponding author gmuca@ubt.edu.al

information gained from the observation of the Neutrophilic presence in the peripheral blood can be used to evaluate the general health status of the animal. Flow cytometry can be considered as one of the techniques that can be used to observe the percentage of different leukocytes populations in different moments after the infection has occurred. Flow cytometry is currently used to for many purposes in veterinary medicine including cell populations identification and immunophenotyping, identification and staging of lymphoproliferative and myeloproliferative neoplastic disorders such as lymphoma and leukemia, detection of minimal residual disease and the detection of various prognostic indexes such as Ki67 (Riondato et al., 2016, Poggi et al., 2017, Sulce et al., 2018, Chaflon et al., 2019, Marconato et al., 2019, Comazzi et al., 2021, Riondato et al., 2021, Rigillo et al., 2021, Riondato et al., 2021, Sulce et al., 2022, Munga et al., 2023). The aim of this study was to observe the Neutrophiles reaction and percentages in the peripheral blood during an induced local infection, through flow cytometry in different moments.

MATERIAL AND METHODS

Five healthy female rabbits were selected for the experiment. All rabbit were New Zealand breed aging six months and having no history of previous diseases. Moreover vaccine against Myxomatosis and Rabbit Hemorrhagic disease were administered at the right moment. Rabbits were purchased at an authorized rabbit farm at 3 month of age and exposed to light for 16h/day. Temperatures ranged from 16 to 25^{0}C while fresh water was always available. Rabbits consumed 140g/day of a standard diet. Rabbits stayed at the adequate facilities of the Faculty of Veterinary Medicine for three months prior to the experiment in order to have the right time to reach their sexual maturity and to adapt their physiological parameters to the place. At time of the experiment the age of the animals was six months.

Rabbits were infected locally (intravaginal administration) with 100 μ/kg body weight *Escherichia Coli* (0127:B8, Sigma–Aldrich, St. Louis, MO, USA) dissolved in 2 mL of sterile saline. The infective dose was not lethal while maximum efforts were made to minimize animal distress. Peripheral blood was collected in different moments: T0 – time when infection was induced, T1 – thirty minutes after infection, T2 – sixty minutes after infection T3 – ninety minutes and T4 – 120 minutes after infection was induced. Flow cytometric analyses took place immediately after all blood collections were performed. Briefly fifty micro liters of each sample was placed in cytometry tubes. An NxT Attune flow cytometer (Thermo Fisher Scientific) was used to perform all analyzes. A lysis step of five minutes was performed to exclude erythrocytes from the analysis and then cells were centrifuged at 1200 rpm for five minutes, supernatant was discarded and another wash using saline buffer was made. At the end cell were acquired to the cytometer in order to produce results. Appropriate gates were designed to analyze only events that are considered as cells excluding debris from the gate of analyzes. Neutrophils percentages were detected each time of acquisition and data were recorded. All analyses took place at the Faculty of Veterinary Medicine, Agricultural University of Tirana.

RESULTS

Results showed that number of Neutrophils in peripheral blood changes depending on time after infection was made. In T0 no movement of neutrophils is observed, while controversially in T1the number decreases significantly, compared to T0. Neutrophils number significantly increases after two hours (T4), showing a general sensitization of these cells in the peripheral blood. During T2 number of neutrophils remains low showing that a specific time is needed for these cells in the rabbit species to increase their number in the peripheral blood after induced infection. Instead during T3 the percentage of Neutrophils begin increasing fast overpassing their percentage in T0. The most representative case is presented in figure 1 in order to understand the movement of the Neutrophilic population in the peripheral blood.

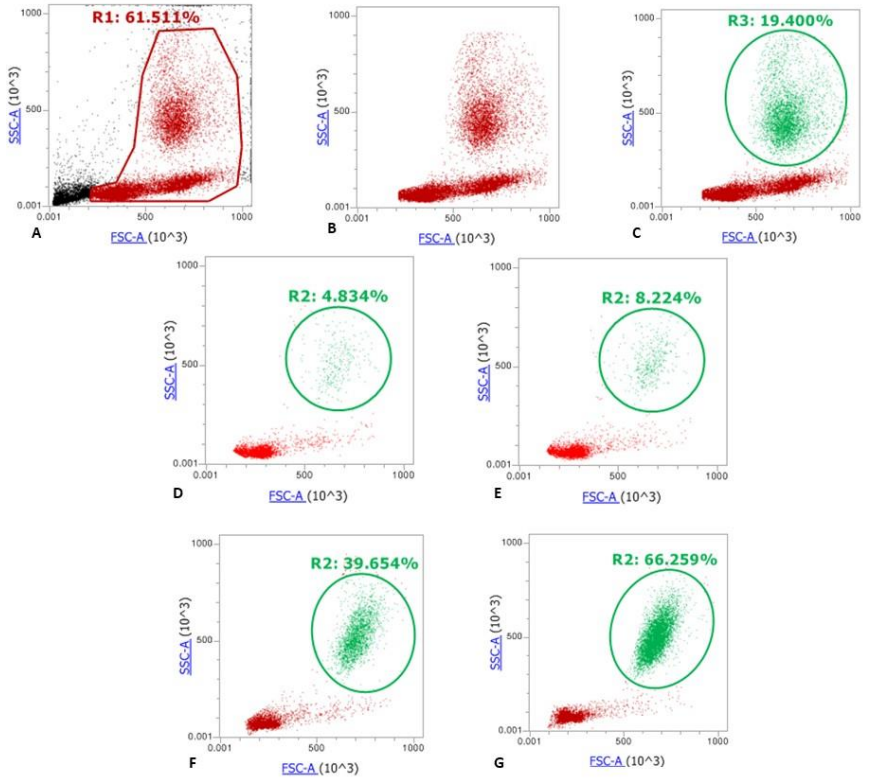

Figure 1. Percentage of Neutrophils in different collection moments after the induced infection. A. Gating strategy to exclude debris from the gate of analyses. B. Designed gate (R1) activated. C. Designed gate to include only Neutrophils in the percentage calculation in T0. D. Percentage of Neutrophils in T1, E. Percentage of Neutrophils in T2, F. Percentage of Neutrophils in T3 and G. Percentage of Neutrophils in T4.

Data regarding the percentages of neutrophils in all five rabbits and in all times of collection after the induced infection are found in table 1.

Table 1.

Animal	% of Neutrophils in T0	% of Neutrophils in T1	% of Neutrophils in T2	% of Neutrophils in T3	% of Neutrophils in T4
1	19.400	4.834	8.224	39.654	66.259
2	31.243	3.113	13.549	42.781	65.259
3	21.693	6.739	10.295	36.936	59.832
4	18.395	5.394	9.429	40.300	57.310
5	24.840	7.356	9.268	45.298	64.192

DISCUSSION

Despite the fact that Neutrofilic movement to the infection site is well known, studies focused on their percentage in different moments after infection through flow cytometry are lacking. This study demonstrated that flow cytometry is a reliable technique to observe the Neutrophil movement in terms of percentages in the peripheral blood of rabbit species. Results from this investigation suggest that in rabbit species the neutrophils move toward the infection site in a considerable short time taking into consideration that in T1 the percentage of these cells is very low in all cases. Almost in all cases the percentage of Neutrophils passes two times their quantity compared to the T0. However this study has several limitation. No markers such as antibodies were used to identify Neutrophiles since we used the scatter properties approach. Moreover the presence of the neutrophils in the infectious site was not proven but only hypothesized. Identifying the active status of neutrophils would be of great interest in future studies in order to demonstrate their preparation for different events that may occur.

CONCLUSIONS

Based on the results gained from this study the flow cytometry appears as a reliable, easily repetitive, fast, accurate and low cost technique to identify the percentages of Neutrophils in rabbit's peripheral blood when an induced infection takes place. Flow cytometry successfully reached to identify Neutrophils even in low percentages showing its capability to provide useful information. However further studies with a higher caseload are warranted to better define the association between differences in Neutrophils percentage in the peripheral blood and the infectious dose.

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Mr. Gerald MUÇA is a lecturer at the Department of Clinical Subjects, Faculty of Veterinary Medicine. During the years 2003-2008, he completed his univeristy studies as a "veterinarian" at the Agricultural University of Tirana. In the years 2009-2011, he completed his Master of Science studies in "Animal Breeding and Reproductive Biotechnologies" organized by the Mediterranean Institute of Saragoza (CHIAM), Spain. He completed his doctoral studies at the Institute of Neurosciences, Alicante, Spain (2012-2020). During the period 2016-2019, he served as a lecturer of the module "Comparative Anatomy" at the Department of Morphofunctional Subjects. He teaches in the Bachelor and Master of Science programs, in the modules "Animal Reproduction", "Biotechnology and Animal Reproduction," and "Clinical Endocrinology". He has published 11 scientific articles, which 11 are in journals with FI or indexed in Scopus, web of science, etc. His scientific studies and publications have been cited more than 49 times by other authors. (https://www.scopus.com/authid/detail.uri?authorId=55923969600)

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