PERITONEAL INFECTION BY *CANDIDA ALBICANS*. STUDY OF NUMBER AND SIZE OF LIMPHOCYTES AND PHAGOCITIC ACTIVITY OF PERITONEAL MACROPHAGES IN MICE[1].

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SUMMARY: The main purpose of this investigation was to study some aspects of leucocytes (granulocytes and limphocytes) and the phagocitic activity of peritoneal macrophages. In this experiment, which took place at Escola Paulista de Medicina - Universidade Federal de São Paulo - Brazil, it was used twenty female C57BLACK mice. Half of them were submitted to radiation to obtain immunossupressed animals (Group A - irradiated mice). The other ten mice were not irradiated (Group B - control). The animals were sorted in four subgroups: A-1, A-2, B-1 and B-2. Mice of the groups A-1 and B-1 were injected with saline, and those of subgroups A-2 and B-2, were infected with Candida albicans (ATCC 90029). The resultant data showed significant differences in the number of leucocytes (granulocytes and limphocytes), and in the medium size of limphocytes between irradiated and non irradiated mice. Related to peritoneal macrophages, it was observed that the number of macrophages was lower in irradiated mice and the phagocitic activity was decreased in the irradiated and infected animals .

SUBJECT HEADINGS: Peritonitis. Candida albicans. Ionizing radiation. Leucocytes. Macrophages.

INTRODUCTION

Although several medical advances have happened in antimicrobial therapy, surgical treatment and surgical intensive care, the number of patients with peritoneal infection remains very high.[3,24,34,37,40]

Recently some authors have described an important role of Candida albicans in mortalility of immunossupressed patients with peritoneal infection [21,22,23,25,31,37,38,40]

Despite of a great number of clinical reports related to peritoneal Candida infections[1,5,18,21,25,29,30,] experimental research is not so numerous.[2, 26]

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Another issue is the role of leucocytes (limphocytes and granulocytes) and peritoneal macrophages in diagnosis of Candida peritoneal infections.[6,7,17,19,36,39]

The purpose of this work was to investigate some aspects of blood cells (number of leucocytes and size of limphocytes) and phagocytosis of peritoneal macrophages in mice, infected by *Candida albicans*.

METHOD

Twenty C57BLACK female mice weighting between 18 e 22 g were used in this experiment. The animals were distributed in two groups of ten mice each one. These groups were irradiated (A) and not irradiated (B). Four subgroups of 5 animals were obtained. The animals of A-1 and B-1 subgroups were intraperitoneally injected with 1,0 ml of physiologic solution and mice of A-2 and B-2 subgroups received 1,0 ml of a solution of 8,8 (±0,5) x 10^5 UFC *Candida albicans**.

Exposure to radiation was performed by a 60 Co ALCIAN-II equipment with the following characteristics: single dose of 500 cGy, whole-body exposure, and an absorved dose of 139,58 cGy.min^{-1}. Animals of A-1 and A-2 subgroups were injected seventy-two hours after the radiation

Ten days after radiation and seven days after injection the mice were submitted to euthanasia and blood analysis was performed in a Coulter T-890 automatic equipment. Slides were prepared to study the size of the limphocytes.

Two mililiters of phisiologic basic solution (PBS) were injected into the peritoneal cavity to recover and prepare the peritoneal macrophages culture.

The number of macrophages were adjusted to 4,5 x 10^5 and incubated in a Costar Tissue Culture Cluster (24 wells). The macrophages culture remained in 5% CO_2 chamber by twenty hours.

Zymozan** was used to study phagocitic activity and Phagocitic Index (PI) was obtained after common light microscope observations. It was counted the number of macrophages that presented zymozan, and the number of zymozan particles in each macrophage. The PI was expressed by percentage of macrophages with zymozan x the number of zimozan particles in each macrophage.

Statiscal analysis were performed by non-parametric tests. KRUSKALL-WALLIS rank variance analysis was used and when there was a stastiscally significant difference it was completed with multiple comparisons tests.The level of significance was 0,05 or 5% (a^3 0,05).

RESULTS

There were many important differences between hematologic data from irradiated and non-irradiated mice. The absolute number of leucocytes and the size of limphocytes were lower in the irradiated groups (1320/1400:5000/3580 by mm^3; 5,44/5,47: 6,51/6,00 micra).

It was observed in subgroup A-2 (irradiated and infected mice) a reduced number of granulocytes when compared with the others subgroups (208/276: 627/413 by mm^3).

On the other side, the PI was also reduced in the animals of subgroup A-2 when compared to B-1 mice (97:300 or 32:100 - in %).

DISCUSSION

The weight of C57BLACK mice were uniform and the known number of *Candida albicans* gave to the experimental design an important caractheristic, an easy possibility of reproduction. *Candida albicans* was chosen based on clinical importance.[13,29,30,31,40]

The known number of Candida and the period post-infection for the study was suggested by many authors.[3,4,8,9,10,15,24,34]

GOMPERTZ (1991)[13] considered a very difficult task to confirm the diagnostic of a *Candida albicans* infection. We could not recover the microrganism in the animals of experiment. Probably, with earlier microbiologic studies this objetive would be reached.

There is a tendency to use mice in some experimental peritonitis research.[2,10,26]

SEGRETO e LUDWIG (1972)[27] e SEGRETO (1992)[28] have studied the depletion of leucocytes in blood marrow in C57 BLACK mice, and observed that the period when the depletion was very severe between 48 and 72 hours after the radiation.

The study of number and size of blood cells could be useful for diagnostic of critical infections.[6,7,17,19,36,39] Some authors[11,14] observed in mice a number of leucocytes between 4000 and 11400/ mm^3. In this study, the averages were between 1320 and 5000/mm^3.

(*) Mc Farland's 0,5 - Number 90029 ATCC (American Type Collection Culture)

(**) *Saccharomices cerevisae*

Based on the many authors' datas[6,7,11,14,16,36,39] we supposed that mice leucocytes were smaller than the human's. The study of the medium size of mice limphocytes was 5,44 to 6,51 micra. The reduction of the size of limphocytes could be related only to radiation or even to radiation and Candida infection in mice.

The phagocitic activity of macrophages can be performed by several methods[12,20,32,33,35]. It was chosen peritoneal macrophages culture and the Phagocitic Index was calculated as previously described.[20,32,35]. This indirect method of observation was a adequate model of phagocitic activity evaluation.[33]

Many recent experimental work could be a reflection of the important role of the macrophages and their phagocitic activity in fungal peritoneal infections, specially by Candida.[12,20,32,33,35] In this way, studying phagocitic activity would be a promising method to improve the survival of patients with peritoneal infection, mainly in those with peritoneal candidiasis.

CONCLUSION

Peritoneal infection by *Candida albicans* in irradiated mice cause a lower number of granulocytes and phagocitosis, while the animals exposed to radiation presents a lower number of leucocytes, limphocytes and a reduction of the size of limphocytes.

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