Comparison of Different Animal Models in the Study of Inflammation

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ABSTRACT: Peritoneal cavity is an excellent in vivo location to inflammation studies. We used three different strains of mice (Balb/c, Black/6 and Charles River) to compare the inflammatory cellular response to talc challenge. The total and the differential peritoneal cell count (namely peritoneal resident cells and inflammatory cells) were studied for a period of 30 days. The highest number of inflammatory cells was observed as early as day 1 in Black/6 mice, but it was only found by the 3rd day post-talc challenge in the other strains of mice. Neutrophils were the first type of cells to arrive at the local, either on the first day in the Black/6 mice and on the 3rd day in the other mice strains. Mononuclear cells predominated on the control group and also for most part of the inflammatory process in challenged animals. During induced inflammation, mast cells were absent, and eosinophils were occasionally observed. A population of multinucleated giant cells appeared on day 1 and reached a peak on different days for the different strains. By day 30th the inflammation remained unsolved in all the strains of mice used in this work. It was also observed that the kinetics of the inflammatory process was different among strains, with distinct cell types being present at different moments of inflammation. Consequently, this work suggests that the purpose of a research could influence the choice of strain.

KEYWORDS: Inflammation, Peritoneal cavity, Talc, Mice

Introduction

Inflammation is the response of an organism to injury. This phenomenon happens to limit and destroy the agent and to restore the healthiness of the affected tissue. The sequence of events that characterize the inflammatory process is mediated by a large number of molecular mediators, some of them pre-existing in the blood or body fluids, while others are produced either by the agent or the damaged cells and tissues after the injury [1]. Metaloproteinase-9 (MMP-9) [2], the complement, Kinins, prostaglandins, thromboxan, immunoglobulins and interleukins, which are chemoattractants to mature leukocytes, are some of those substances. Recruitment of these cells into inflamed tissues by the process called extravasion is a prominent feature of acute and chronic inflammation [3]. Neutrophils are the first cells that migrate and accumulate at this site [3]. The predominance of macrophages, eosinophils, mast cells, or lymphocytes in the local of inflammation depends on several factors, concerning a complex phenomenon that shows individual variations.

In this work we propose to follow and compare the kinetics of a talc-induced peritoneal inflammation in three different strains of mice for a 30 days period. In addition, we aim to determine if a particular mouse strain should be considered a variable criterion requesting attention when designing a peritoneal inflammation model.

Materials and methods

Animals

Balb/c, Black/6 and Charles River strains of mice, purchased from Harlan Iberica, were used. Animals, aged of 6-8 weeks, were and kept under regular standard manipulation conditions and hygiene (according Portuguese and European guidelines for care and handling of laboratory, and the project have been approved by the National Veterinary Directorate), fed with a commercial chow and given acidified drinking water ad libitum. Animals from each strain of animals were divided in 10 groups, each one composed of 10 animals. For every strain, each one of the ten groups corresponded to a different time of inflammation.

Induction of inflammation:

The intraperitoneal inflammation was induced through inoculation of 200ºl of a sterile 10% aqueous talc suspension.

Cell collection

For each strain, at predefined times over a 30 days period (day zero, or control, day 1, 2, 3, 5, 7, 9, 12, 16 and 30), a group of animals was sacrificed, and the...
peritoneal cells were collected within cold PBS (phosphate-buffered saline at pH=7.4), and differential cell counts were performed in cytopsins preparations (using a Cytospin® 3 - Shandon®), and afterward stained with Wright's stain.

**Cell count**

After collection, peritoneal cells were counted on a Neubauer chamber. On Wright stained cytopsins, at a 400x magnification, the total and the differential cell count on a total of 1000 cells were determined for each animal. Cells were classified as: mononuclear cells (macrophages, mesothelial cells and lymphocytes), neutrophils, eosinophils, mast cells, binucleated cells and multinucleated cells.

The statistical analysis was performed with Statview®, using the t-student test.

**Results**

On day zero, Charles River showed a higher number of peritoneal resident cells (693x10⁴cell/ml), when compared to the other studied strains. By contrast, Balb/c showed the lowest cell number (314x10⁴cell/ml for Balb/c and 466x10⁴cell/ml for Black/6). It was also noticed that the mononuclear cells were the most prevalent cell type in all the strains, while neutrophils were only occasionally found. Mast cells, eosinophils and binucleated cells were present in control animals, although in small counts (Graph 1 to 3). On the first day of the inflammation, neutrophils were the predominant cell type in all the studied strains of mice (398x10⁴cell/ml for Balb/c, 771x10⁴cell/ml for Charles River and 995x10⁴cell/ml for Black/6). Black/6 revealed a peak of neutrophils on day 1 post-challenge, although a similar peak was only reached on the 3rd day after tail inoculation in the other two strains. Neutrophils number diminished significantly after day 5 in Charles River and Black/6 strains, but decreased only by day 7 in Balb/c mice. After day 12 of the induced inflammation, these cells became residual.

In controls (day zero), mononucleated cells were the predominant cell type in the peritoneal cavity: 294x10⁴cell/ml for Balb/c, 592 x10⁴cell/ml for Charles River and 417x10⁴cell/ml for Black/6. Despite an initial reduction on its number observed at the day 1 of inflammation, mononucleated cells duplicated in number after day 3. Mononucleated cells are the predominant cell type in the peritoneal cavity during the entire inflammatory process, and they appeared subsequently to the peak of neutrophils. They reached a peak on day 3 in Charles River and Black/6 strains, but only at day 7th in the Balb/c strain. From this day on, the mononucleated cells diminished below control values and remained low until day 30th.

A small amount of eosinophils were present in the control group: 9.4x10⁴cell/ml in Balb/c, 7.6 x10⁴cell/ml in Charles River, and 19.7 x10⁴cell/ml in Black/6. Despite an initial reduction on its number observed at the day 1 of inflammation, mononucleated cells duplicated in number after day 3. Mononucleated cells are the predominant cell type in the peritoneal cavity during the entire inflammatory process, and they appeared subsequently to the peak of neutrophils. They reached a peak on day 3 in Charles River and Black/6 strains, but only at day 7th in the Balb/c strain. From this day on, the mononucleated cells diminished below control values and remained low until day 30th.

Graph. 2 - Charles River cells during the inflammation.

Legend: MN - mononuclear cells; PMN - neutrophils; EOS - eosinophils; Bin - binucleated cells; CGM - multinucleated giant cells; Mast - mast cells; TOT - total number of cells.

Graph 1 - Balb/c cells during the inflammation. Legend: MN - mononuclear cells; PMN - neutrophils; EOS - eosinophils; Bin - binucleated cells; CGM - multinucleated giant cells; Mast - mast cells; TOT - total number of cells.

Graph. 3 - Black/6 cells during the inflammation.

Legend: MN - mononuclear cells; PMN - neutrophils; EOS - eosinophils; Bin - binucleated cells; CGM - multinucleated giant cells; Mast - mast cells; TOT - total number of cells.

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Binucleated cells increase in number by day 7th in Balb/c strain, although in the other strains no such difference was found throughout the inflammatory process.

Multinucleated giant cells (MGC) were only found in Charles River controls (11x10^4 cell/ml), being absent in Balb/c and Black/6 controls. In Balb/c strain, MGC were found after the 1st day of inflammation, reaching a peak by day 7th (10x10^4 cell/ml). In Black/6 strain, the peak of multinucleated giant cells was reached by day 3rd (17x10^4 cell/ml), while in the Charles River it was only observed by day 7th (20.5x10^4 cell/ml).

A small population of mast cells was found in the controls' peritoneum, in a number comparable to the observed for eosinophils and binucleated cells (7.5x10^4 cell/ml for Balb/c, 22.3x10^4 cell/ml for Charles River and 16.2x10^4 cell/ml for Black/6). During the peak of inflammation, mast cells disappeared from the peritoneal cavity. In Balb/c they returned in reduced numbers on day 5th (3.8x10^4 cell/ml), but only on day 16th and 30th in Charles River (with 6.4x10^4 cell/ml, and 8.5x10^4 cell/ml, respectively), and on by day 12th in Black/6 strain (3.8x10^4 cell/ml).

Discussion

Results from the animal models used in this work showed that neutrophils were the first cell type to migrate into the inflammatory local, what is accordance to other reports [3]. Our work registered the existence of strain variations in the moment of arrival of cells and in the number of cells detect in the local of inflammation: the peak of leukocytes was first observed in Black/6 at day 1, but only at day 3 in Balb/c and Charles River. These findings could be explained by the existence of different mediators in the peritoneal cavity or by the resident cells ability to produce larger quantities of neutrophils' chemokine factors. Macrophages and mast cells play an important role in releasing pro-inflammatory products, as histamine, interleukins, prostaglandins and leukotrienes, which regulate peritoneal vascular permeability. Aujchen and colleagues [4] and Kolaczkowska and collaborators [2] showed that peritoneal mast cells and macrophages are important players in acute phase response, driving neutrophils chemotaxis to the inflammation area. MMP-9 is a factor recently demonstrated to be associated to neutrophil infiltration of the inflammatory focus [5]. It is produced mainly by neutrophils, although macrophages, mast cells, lymphocytes, dendritic cells, fibroblasts and tumour cells may also contribute for its production [6].

Unstimulated peritoneal mast cells constitutively present MMP-9 and macrophages produce it since initial stages of inflammation, as it has been demonstrated by Kolaczkowska and collaborators [2,7]. Although some reports defend that the presence of mast cells does not influence the normal course of inflammation [2], our data did not allow to associate mast cells presence with inflammation outcome in the peritoneal cavity due to the fact that its presence during the inflammatory process was inconstant and seemed highly dependent on the strain.

Data obtained in this study show that macrophages are the predominant mononucleated cells at day zero; nevertheless, these cell number decreased soon after the inflammatory stimulus, although it duplicated from day 3rd on. Fluctuations in the number of mononucleated cells were observed, in respect with time and mice strain. Not even after 30th days post-inoculation they reach the values of control. These results are in accordance with data obtained in lung acute inflammatory processes, were monocyte accumulation usually follows that of the neutrophils [8]. Doherty and colleagues [8] showed that monocyte accumulation in several systems is a neutrophil-dependent event, probably because neutrophils generate monocyte chemotactants factors; it has also been demonstrated that neutrophil population may differ with the inflammatory stimulus [1].

Macrophages play a critical role in inflammation; together with their function in the onset and the resolution of an inflammatory lesion, they also constitute the first-line of defending leukocytes [2].

Data obtained in this study demonstrated that eosinophils and binucleated cells (resulting from the fusion of macrophages or mesothelial cells) constitute a residual cell population at day zero despite that strain variations were observed. Binucleated cells did not show significant variations in the talc-induced inflammation. In all the mice strains eosinophils reached an insignificant peak on day 3rd of inflammation. Das and colleagues [9] defend that in control animals mast cells existence could be related with the residual eosinophils population.

During inflammation, the eosinophils peak could be associated to factors produced by macrophages or lymphocytes, as RANTES [9], IL4 or IL-5 [10].

MGC are cells with 3 or more nuclei resulting from the fusion of recently arrived macrocytes from blood [11]. They show distinct morphology according to the different stimulatory factors, and are typical for some types of chronic inflammation and granuloma. Whereas these cells were not detected in Balb/c and Black/6 at time zero, they were found in Charles River controls, albeit in very low numbers. They increased after the day 1 of inflammation, reaching a peak by day 3 in Black/6 and by day 7 on Balb/c and Charles River. The phagocytosis of a foreigner substance simultaneously by several macrophages in a solid surface has been hypothesising to be the initial step for macrophage fusion. In addition, surface molecules, like MHCI [12], CD44 or CD47 [11, 12] and IL-6 were also proved to be important to macrophage fusion. In the talc-induced inflammation, only scarce number of MGC, showing a low number of disorganized nuclei was detected. This could be consequence of the inexistence of a solid surface in the peritoneum (the cells are in suspension) or to the lack of certain interleukins in this type inflammation, which would restrain MGC formation [12].

In conclusion, our studies demonstrate that the inflammatory cells kinetics are dependent of the strain of mice used: the acute phase response of inflammation is more rapid in the Black/6 mice, while the study of
chronic inflammation with promotion of MGC formation is more accurate in Balb/c mice than in the other two strains. To minimize variations, the ideal model would use inbred animals. Nevertheless, even for that it would be desirable to know the particularities of each strain and correlate them with the aim of the project. As we see, the variations between strains can conditioned the final results, and the knowledge of normal values of each one is useful to the choice strain model.

References