

## **Evaluation of immune response of BALB/c to homeopathic solutions**

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### **ABSTRACT**

**Introduction:** Biotherapies are medicines prepared from etiologic agents, following Brazilian Homeopathic Pharmacopeia. Influenza is a disease that affects thousands of people worldwide every year, motivating the development of new therapies.

**Aim:** In this study, we developed two biotherapies from live/active influenza A virus, at 12x and 30x, and verified some immune response parameters in mice.

**Methodology:** The biotherapeutic was administered to male SPF 4 weeks old Balb/c mice. The protocol was approved by the UFRJ Ethics Committee of Animal Use (Protocol DFBCICB 040). Animals were stimulated daily, blindly, with different homeopathic medicines, at 1% (V/V) for a maximum period of 42 days. Three homeopathic medicines were tested: biotherapeutic 30x containing active influenza A virus; biotherapeutic 12x containing active influenza A virus; and thymulin 5cH. The experimental groups were: Group A (5 animals) – administration of thymulin 5cH, Group B (5 animals) – administration of biotherapeutic 30x, Group C (5 animals) – administration of biotherapeutic 12x, Group D (5 animals) - administration of a water 30x, Group E (5 animals) - administration of a water 12x, Group F (5 animals) - control (without treatment). After 21 days of treatment, all animals were challenged subcutaneously with the viral hemagglutinin antigen at the concentration of 7 µg/200µL and monitored by further 21 days. After euthanasia, all animals were autopsied and the spleen was collected for weight and immunohistochemistry analyses. Additionally, peritoneal washing was done and a “pool” of samples from each group was prepared to be analyzed by flow cytometry.

**Results:** Mice treated with biotherapeutic 30x and thymulin 5cH showed similar profile, different from controls, in which a switch of lymphocytes/phagocytes proportion in the peritoneum was seen, followed by predominance of B1b cells in relation to conventional B and T cells ( $X^2$ ,  $p=0.005$ ). Regarding to T cell population, in the contrary to control, CD4+ cells were predominant in relation to CD8+ cells ( $X^2$ ,  $p=0.0001$ ). The immunohistochemistry revealed increase in the number of activated CD11b+ macrophages in spleen ( $p<0.05$ ), but no difference were detected in the spleen lymphocytes profile.

**Conclusion:** The results show that the action of biotherapeutic 30x and thymulin 5cH have similar immune modulation effects, improving the innate immune response.

**Key-words:** Biotherapeutic, Influenza, flow cytometry, immunohistochemistry, animal model.

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