Study of the Interferonogenous Activity of Del-Immune V®


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Among a large number of presently known therapeutic products based on probiotic bacteria, Del-Immune V® is enjoying growing popularity. This formulation is a dry enzymatic lysate of the cells of a probiotic strain of *Lactobacillus rhamnosus DV*. As an accompanying preparation, Del Immune V® is highly effective in viral (flu, hepatitis C), bacterial (bronchitis), and fungal diseases, allergies, asthma, chronic fatigue, fibromyalgia, etc. However, the mechanism of the wide-spectrum biological activity of this formulation needs to be explained.

In order to clarify the mechanisms of Del-Immune V® biological activity, a study of its capacity to produce cytokines such as interferon (IFN) and tumor necrosis factor (TNF) was studied *in vivo* and *in vitro*.

**Materials and Methods of Research**

The effect of Del-Immune V® on the immune response of the body was studied in nondescript mice weighing 14-16 g. These animals selected by analogy were divided into 5 groups of 16 each. Groups I-III were administered 0.5 ml of an aquatic solution of Del-Immune V® *per os* via olive-tipped needle every 24 hours for 5 days. In groups I-III, specific doses of 5, 50 and 500 mcg/mouse were administrated respectively. Group IV mice were administered 0.5 of aquatic suspension of Bifidim (control probiotic formulation) for 5 days at 50 mcg/mouse. Group V mice were administered 0.5 ml of a physiological saline solution. IFN and TNF production was studied in both intact and experimental animals 8, 24, 72 and 120 hours after administration of the respective formulations. For this, blood serum, spleen, and peritoneal exudate macrophages (PEM) were tested.

Cells (1x10^7 cells/ml) were cultivated in 24 Falcon test tubes. The inductors were given in 5-500 microgram doses. Evaluation of the interferon capacity of the preparations researched was carried out according to standard inductors (for IFN-α, the Newcastle Virus was used. For IFN-γ, 20 mcg/ml of PHA was used (Difco).

In splenocytes and PEM (1x10^7 cells/ml) of intact and experimental mice, cytokines were induced: IFN-α for the Newcastle virus (NVD) at a multiplicity of 10 TCD 50/cell, and PHA (20 mcg/ml, Difco) for the induction of IFN-γ. The activation capabilities of Del-Immune V® and Bifidim in concentrations of 5, 50 and 500 mcg/ml were also studied.

IFN and TNF levels were defined 6, 24 and 48 hours after cell incubation with the formulation in the culture media. The biological activity of TNF was determined by the cytotoxic activity of the fibroblast mouse culture l-929 (Malaytsev, 1991). Measurements were carried out on a cytotoxic standard index employing a calibrated curve constructed on the basis of a recombination of INF “Sigma.”

The level of IFN in the cell culture and blood serum was determined by a standard microtitration of the fibroblast mouse culture l-929 against 100 TCD50 virus inductor (VVS Indiana Virus) at constant CO₂ levels. For statistical evaluation, Student’s criteria was used.

**Results and Discussion**

Daily administration of Del-Immune V® or Bifidim for 5 days in a dose of 50 mcg/ml resulted in a marked increase of INF level in the blood serum of the experimental animals. In a 24 hour observation of the IFN level a 4-5 log₂ was achieved. Upon subsequent administration, a 5.5±0.7 log 2 U/ml was achieved. Further administrations of Del Immune V® at
doses of 50 mcg/ml over a 3 day period provided maintenance of the achieved level of 5.5±0.5 U/ml. Administration of the preparation over a 4 day period led to some reduction in the level of circulated IFN. An analogous result was observed with the administration of Del Immune V® in doses of 5 and 500 mcg/ml (Figure 1).

The introduction of the preparation of Del-Immune V® or Bifidim in doses of 5, 50, 500 mcg/mouse led to the development of endogenous TNF. The index of cytotoxicity of TNF upon oral administration of Del-Immune V® achieved a corresponding 15±1.8% and 14±1.7% (P<0.05), in the control group 8±1.2%. The maximum production of TNF in the blood serum of tested mice took place 8 hours after administration of the preparation (Figure 2).
Administration of Del-Immune V® in doses of 5mcg/mouse did not influence the production of TNF. Dosage increases up to 500 mcg/mouse increased the cytotoxic index 12±2.3%, compared to 8% in the control group.

The enculturation of mouse splenocytes that received experimental preparations with the inductors (Newcastle virus and PHA) led to a two-fold increase in interferon response compared to the cells of the control mice (Figure 3).
Figure 3. The interferonogenous activity of mouse splenocytes upon the introduction of Del-Immune V® and Bifidim
Consequently, the experimental preparation positively influenced the immune response evaluated by interferon levels. IFN status was determined by the nonspecific resistance of the mice. Other measured indicators included IFN levels in the circulating blood, level of IFN-α and IFN-γ production in vitro stimulation of immune cells and spontaneous IFN levels.

Del-Immune V® and Bifidim each produce distinct dose response curves. We observed spontaneous IFN, IFN-α and IFN-γ levels to be significantly higher with Del-Immune V®.

This capability to increase production of IFN-α and IFN-γ under the influence of corresponding INF inductors was observed in splenocytes after 8, 24 and 72 hours following introduction of the experimental preparations.

In this manner, the introduction of the probiotic preparations Del-Immune V® and Bifidim in optimal doses stimulates the IFN production and creates an increase in effectiveness of other inductors of interferon. Therefore, we have researched the interferon activity of these preparations in vitro.

Macrophage cell cultures obtained from animals treated with Del-Immune V® and Bifidim demonstrated cytokine IFN and TNF synthesis (Figure 4).

**Figure 4. Interferon activity of Del-Immune V® and Bifidim in the microphage culture of intact and experimental animals**

![Graph showing interferon activity comparison between Del-Immune V® and Bifidim in different doses and time points for testing and control animals.](image-url)
The introduction of Del-Immune V® or Bifidim in doses of 5, 50 and 500 mcg/ml in the macrophages resulted in synthesis of IFN. An even higher level of interferon was observed in the first 24 hours of the cultivation of cells with the research preparations. The macrophages from control mice produced significantly less IFNs.

The introduction of Del-Immune V® and Bifidim in doses of 5, 50 and 100 mcg/ml to the macrophages of experimental and intact control animals led to the development of TNF endogens. The maximum level of TNF was checked 6 hours after the introduction of the preparations (Figure 5).

![Figure 5. Production of TNF under the influence of Del-Immune V®, Bifidim and LPS in the macrophage culture of experimental and intact mice](image)

The administration of Del-Immune V® and Bifidim was repeated in group of test animals following a rest period. Macrophages collected from these animals produced significantly higher TNF levels than control mice.

**Summary and Conclusion**

The lysed probiotic preparation Del-Immune V®, in optimal doses, demonstrates high levels of interferon induction activity both in vitro and in vivo. Del-Immune V® influences the production of TNF factor. An additional advantage is oral ingestion.

The experimental results of these studies allow for the following conclusions:

1. Del-Immune V® demonstrates a high level of interferon activity both in vitro and in vivo (in cell cultures), and in oral administration in experimental animals. Interferon production levels were raised 2.5 to 3 times. The TNF production level was raised 2 times compared with the control animals.
2. The administration of Del-Immune V® leads to an immediate immune response manifesting a high level of interferon, after the first 8 hours. The first levels of TNF manifest themselves an hour after administration and reach maximum levels in 8 hours. A decrease in the control level occurs at the end of the 3rd 24 hour period following a single dose.

3. The maximum level of interferon production was observed on the second 24 hour period, was maintained for 3 days, and on the 4th day decreased only slightly.

4. Repeated administration of the preparation led to an enhanced immune response, with higher levels of interferon production, which increased 50-75%, depending on the dose of the preparation. An optimal dose of Del-ImmuneV®, 50 mcg/ml, provided the maximum level of interferon production. In doses of 5, 100 and 500 mcg/ml the response remained 20-30 percent lower than the maximum, but still higher than the control.

5. The TNF factor increased 10 times higher than the control group. Optimal doses of 100 mcg/ml creates 1.5 ng/ml of TNF compared to control (0.16ng/ml). Repeated administration lead to an TNF increase of 20-25%.

6. In this research, orally administered Del-Immune V® demonstrated a broad spectrum of immune modulation properties. The product showed potential for clinical application when used alone or as an adjunctive therapy.

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