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# BACTERIAL IMMUNOSTIMULANT (BRONCHO-VAXOM) VERSUS LEVAMISOLE ON THE HUMORAL IMMUNE RESPONSE IN MICE.

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## ABSTRACT

A comparative study on the enhancement of humoral immune response in mice after oral treatment with levamisole or a lyophilized bacterial lysate (Broncho-Vaxom) is presented. The latter proves to be more effective at therapeutic doses than levamisole on the induction of immunoglobulin formation and particularly that of IgA in secretions.

## INTRODUCTION

Levamisole is a levorotatory enanticmer of the low molecular weight tetramisole (2, 3, 5, 6 - thetrahydro-6-phenylimidazo |2.1 - 6| thiazole). Although this drug was originally developed as an anti-helminthic for veterinary and human medicine (1) it also acts by stimulating cellular immune responses in various animal species, including man (2, 3, 4). Different authors have furthermore demonstrated its efficiency in the treatment of several immunodeficiency syndromes (5, 6, 7).



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Broncho-Vaxom is a lyophilized bacterial lysate prepared from eight different bacterial species frequently associated with respiratory diseases. This bacterial preparation induces an specific and inespecific cell-mediated immune response in man (8). Moreover in healthy volunteers, Broncho-Vaxom stimulates the production of secretory IgA in saliva and of IgG and IgM in serum (9) and compared with levamisole, potentiates the lymphocytic response (10).

The purpose of the present experiment was to compare the respective effects of two immunostimulants, one of synthetic origin (levamisole) and the other of bacterial origin (Broncho-Vaxom), on the humoral immune response, particularly on secretory and seric IgA and IgG.

## MATERIAL AND METHODS

### Mice

Six to eight weeks old female swiss mice were obtained from the "Instituto Central de Animales de Laboratorio", Daganzo, Spain.

### Treatments

Two different treatments with levamisole (Janssen Pharmaceutica, Beerse, Belgium) were scheduled. During 10 days, 30 mice were daily given and oral dose of 25 μg of levamisole diluted in 0.2 ml of saline (L treatment): during the same period, 30 other mice received daily 250 μg of the drug in similar amount of saline (10 L treatment).

Concerning the Broncho-Vaxom treatment (OM Laboratories, Meyrin-Geneva, Switzerland), 30 mice received daily for 10 days an oral dose of the immunobiotherapeutic preparation diluted in 0.2 ml of saline (B treatment). This preparation contains 2.33 mg per ml of a lyophilized bacterial lysate of Haemophilus influenzae, Streptococcus pneumoniae, Klebsiella pneumoniae and Klebsiella ozanae, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans

and Neisseria catarrhalis; 10 control mice were daily given 0.2 ml of saline for 10 days.

Ig determinations

At different intervals after the beginning of the treatments, the mice were sacrified by cervical dislocation and their gut, trachea and lungs were removed and placed at 4°C. These organs were then thoroughly rinsed with saline containing 1‰ solution of sodium azide. Their total content and rinsing solution were twice centrifugated at 8000 x G and 100 - fold concentrated by negative pressure ultrafiltration using 8/32 Visking tubing membranes (Scientific Instruments Center Ltd., London, England), and kept frozen until use. Blood samples were collected by cardiac puncture and the sera kept frozen until the Ig assay. IgA, IgG1 and IgG2a determinations were performed by quantitative radial immunodiffusion (11) using commercially available plates (Meloy Lab. Inc. Springfield, Virginia, U.S.A.). In order to obtain the amount of Ig per mg of protein, the protein content of the gut and pulmonary samples previously concentrated was determined by the Lowry method.

## RESULTS

Igs in gut and pulmonary secretions

The effects of Broncho-Vaxom and levamisole in mice were compared to untreated controls as a ratio. IgG values represent the addition of IgG1 and IgG2a values.

The data from intestinal secretions (Figure 1) reveal significantly ( $p < 0.05$; Student's t test) higher levels of IgA in mice that received Broncho-Vaxom, compared to those treated with levamisole, particularly at therapeutic doses.

The difference in the IgA inducing efficiency of the treatments under study appears to be clear also in pulmonary secretions (Figure 2). Levamisole treated mice have a much weaker humoral response

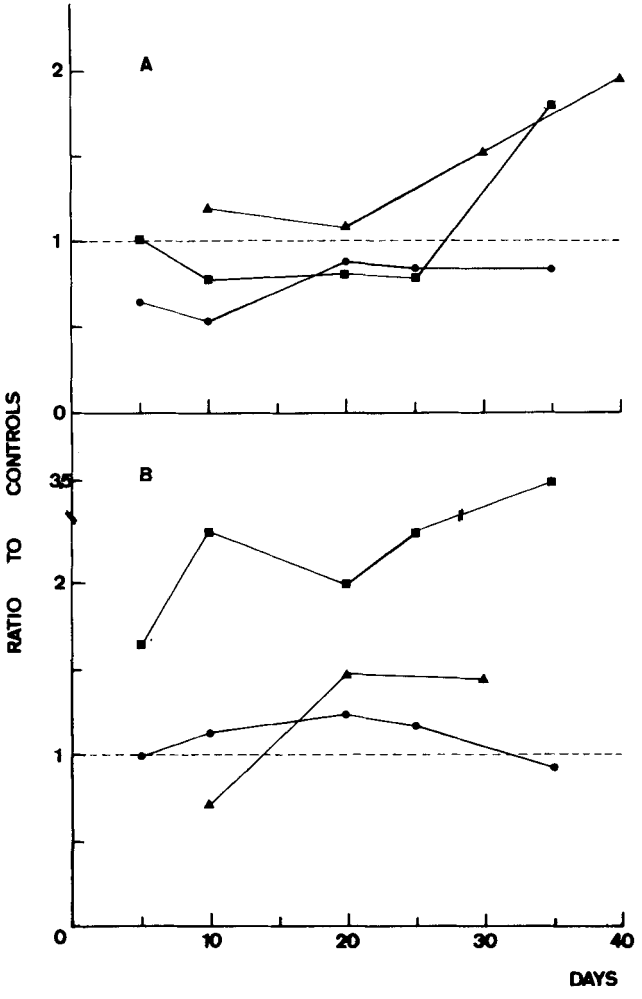


FIG. 1.- Effect of the oral administration of Broncho-Vaxom and levamisole on the levels of IgA (A) and IgG (B) in gut secretions. The effects of the agents are compared to the controls. ▲—▲ Broncho-Vaxom; ●—● levamisole (250 ug); ■—■ levamisole (2500 ug).

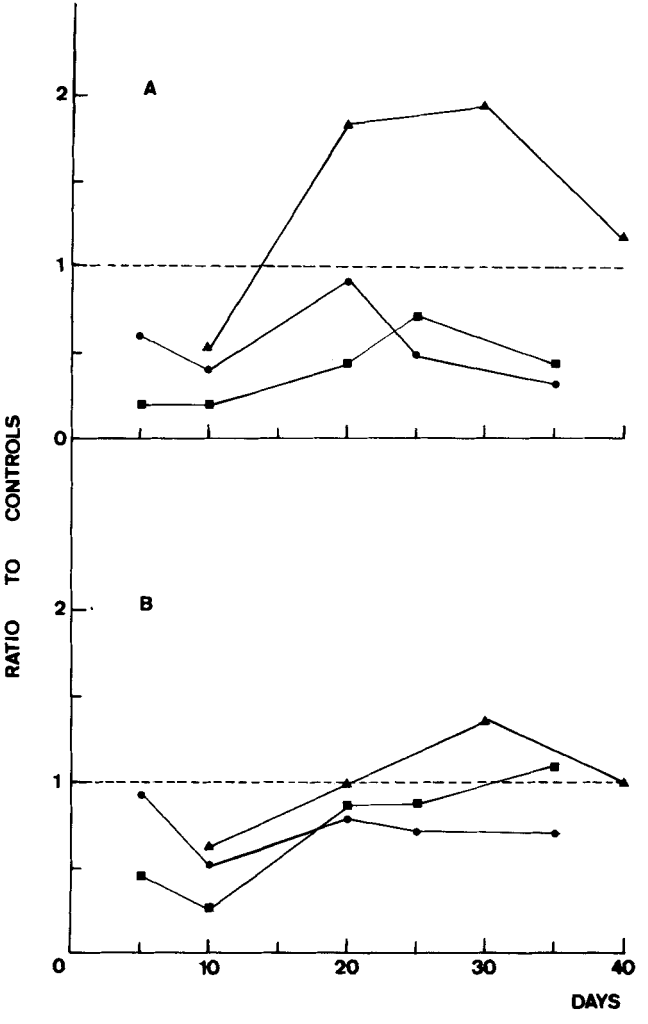


FIG. 2.- Effect of the oral administration of Broncho-Vaxom and levamisole on the levels of IgA (A) and IgG (B) in lung secretions. The effects of the agents are compared to the controls. ▲—▲ Broncho-Vaxom; ●—● levamisole (250 ug); ■—■ levamisole (2500 ug).

than Broncho-Vaxom-treated mice. The latter show a significantly ($p < 0.05$; Student's t test) higher increase in the levels of IgA than do levamisole treated mice, most of which fail to express increments in the Ig levels.

Seric Igs

The results from serum samples (Figure 3) suggest a significantly ($p < 0.05$; Student's t test) higher efficacy of Broncho-Vaxom treatment in front of levamisole, at therapeutic doses (L treatment) in the IgA inducing response. Neither Broncho-Vaxom nor the L treatment of levamisole seem to be capable of inducing seric IgG formation. Only very high doses of levamisole (10 L treatment) are effective in the enhancement of IgG response in serum.

## DISCUSSION

Levamisole seems to be efficient in the regulation of cell mediated immune reactions by restoring effector function of periferal T-lymphocytes and phagocytes, and by stimulating precursor T-lymphocytes differentiation into mature cells (2, 3, 4, 13). It has also been reported to increase plaque forming cell production in adult and aged mice (2). However our results suggest that levamisole has little effect on the humoral immune response in mice. Levamisole induces a very weak response on the immunoglobulins present in secretions and is only active on seric IgG, but at doses too high to be recommended for oral treatments. Accumulated evidence (4) demonstrates that therapeutic doses of levamisole do not influence B-lymphocytes but only enhance the monocytic chemotaxis of T-lymphocytes, which may explain the weak effect of levamisole at the humoral level.

*On the other hand, mice orally treated with Broncho-Vaxom* showed a significant enhancement of the humoral immune response, particularly that of Igs in secretions. In a bacterial lysate such

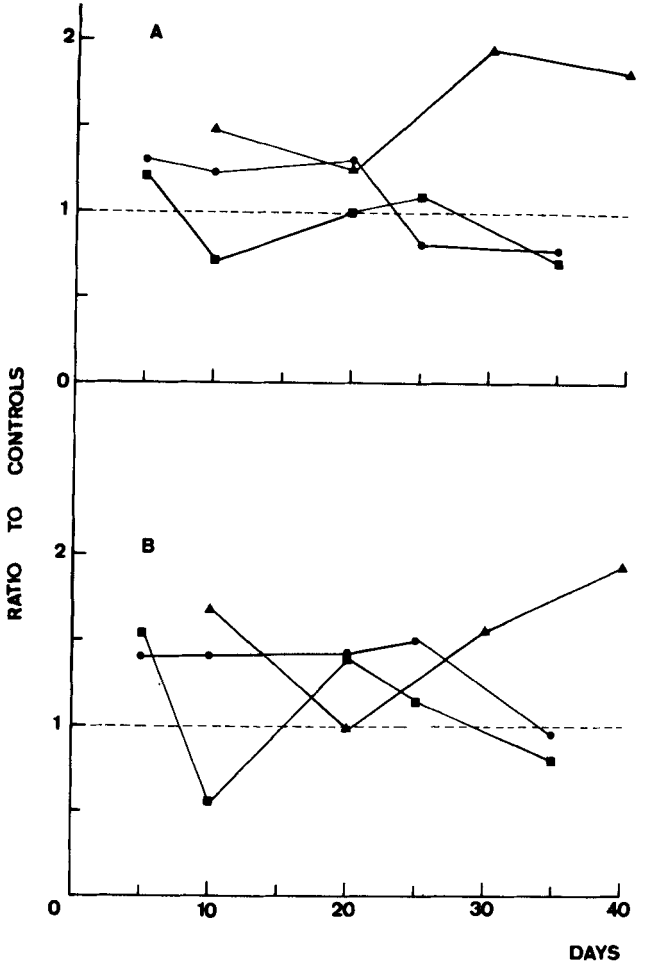


FIG. 3.- Effect of the oral administration of Broncho-Vaxom and levamisole on the level of seric IgA (A) and IgG (B). The effects of the agents are compared to the controls. ▲—▲ Broncho-Vaxom; ●—● levamisole (250 ug); ■—■ levamisole (2500 ug).

as Broncho-Vaxom there is a mixture of molecules that may act either as immunopotentiators or as antigens. Although the increase in IgA levels is of a magnitud enough to suggest that a polyclonal activation occurs, no conclusions can be drawn out on the specificity of such IgA induction from the experiments herein described. No information is available on the effectors to immunomodulators present in mucosae.

Provided that the drug behaves as an immunostimulator at the mucosal level, the increase in IgA both in gut and respiratory tract secretions after oral administration is expectable since it has been proved that the various mucosae, including gut and respiratory tract, act integrated by an extensive system of migratory IgA precursor cells, which stimulated in one of such mucosae are commited to produce IgA in all of them (14, 15, 16, 17, 18, 19, 20). The increase in Igs observed in secretions may or not occur in serum too after stimulation at the mucosal level (14, 18).

A great deal of importance has been given to IgA as one of the main barriers against infections through mucosal surfaces (16, 17, 18, 20). Since Broncho-Vaxom has been described to increase the levels of IgA in secretions in normal man as well as in immunosuppressed and normal mice (21), and at the same time, to decrease recurrence of respiratory diseases in IgA deficient children (8), it can be concluded that Broncho-Vaxom has a therapeutic value in the prevention of infections through respiratory mucosal tissues.

## REFERENCES

1. Thienpont, D., Vanparijs, D.F.J.Z., Raeymaekers, A.M.H., Vandenberk, J., Demoen, P.J.A., Allewijn, F.T.N., Marsboom, R.P.H., Niemegeers, C.J.E., Schellekens, K.H.L.Z. and Janssen, P.A.J. Tetramisole (R8299) a new potent broad spectrum anthelminticum. Nature, 209: 1084, 1966.

2. Renoux, G. Modulation of immunity by levamisole. Pharmac. Ther. 2: 397, 1978.

3. Renoux, G. and Renoux, M. Immunostimulation par le lévamisole: cibles et mécanismes. La Nouvelle Presse médicale, 7: 197, 1978.

4. Symoens, J. Rosenthal, M., De Brabander, M.Z. and Goldstein, G. Immunoregulation with levamisole. Springer Semin. Immunopathol. 2: 49, 1979.

5. Basch, C.M., Spitler, L.E., Engleman, E.G.Z. and Engleman, E.P. Cellular immune reactivity in patients with rheumatoid arthritis and effects of levamisole. J. Rheumatol. 4: 377, 1977.

6. Delespesse, G., Vrijens, R., Demaubeuge, J., Hudson, D., Kennes, B.Z. and Govaerts, A. Influence of levamisole on the immune response of old people. Int. Archs. Allergy appl. Immun. 54: 151, 1977.

7. Lieberman, R. Levamisole-mediated restoration of cellular immunity in peripherial blood lymphocytes of patients with immunodeficiency diseases. Clin. Immunol. and Immunopathol. 5: 142, 1976.

8. Clot, J.P. and Andary, M. Immunostimulation induite par un lysat bactérien lyophilisé. Etude in vitro des réponses spécifiques et non spécifiques. Méd. et Hyg. 38: 2776, 1980.

9. Puigdollers, J.M., Rodes, G., Hernández del Rey, I., Tilló, M.T. and Jofre, J. Immunoglobulin production in man stimulated by an orally administered bacterial lysate. Respiration, 40: 142, 1980.

10. Girard, J.P. and Fleury, S. Analyse comparative du lévamisole et d'un lysat bactérien sur la réponse lymphocytaire in vitro. Méd. et Hyg. 37: 2519, 1979.

11. Mancini, G., Carbonara, A.O.Z. and Heremans, J.F. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2: 235, 1965.

12. Lowry, O.M., Rosebrough, N.J., Farr, A.L.Z. and Randall, R.J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265, 1951.

13. Hadden, J.W., Coffey, R.G., Hadden, E.M., López-Corrales, E.Z. and Sunshine, G.H. Effects of levamisole and imidazole on lymphocyte proliferation and cyclic nucleotide levels. Cell. Immun. 20: 98, 1975.

14. Bienenstock, J. and Befus, A.D. Mucosal Immunology. Immunology, 41: 249, 1980.

15. Bienenstock, J., Befus, A.D. and McDermont, M., in The Mucosal Immune System, edited by F.J. Bourne, p. 5, Martinus Nijhoff Publishers, 1981.

16. Lamm, M.E. Cellular aspects of immunoglobulin A. Adv. Immunol. 22: 223, 1976.

17. McGhee, J.R., Mestecky, J. and Babb, J.L. Secretory immunity and infection. Adv. Exp. Med. Biol., I: 107, 1978.

18. McNabb, P.C. and Tomasi, T.B. Host defense mechanisms at mucosal surfaces. Ann. Rev. Microbiol. 35: 477, 1981.

19. Porter, R. and Knight, J. Immunology of the Gut. CIBA Foundation Symposium 46. Elsevier. Excerpta Medica. Amsterdam, 1977.

20. Tomasi, T.B., in The Immune System of Secretions, edited by A.G. Osler and L. Weiss. Prentice Hall Inc. Eglewood Cliffs, New Jersey, 1976.

21. Bosch, A., Lucena, F. Parés, R. and Jofre, J. Animal experiments on the compensation of the immunosuppressive action of cyclophosphamide by a bacterial derivate (Broncho-Vaxom). Immunobiology, 160:9, 1981.