

CARTAS AL EDITOR

Effect of *Phaseolus vulgaris* L. Isolectins on the Mitogenic Activity of Lymphatic Ganglia Lymphocytes in Rats

It is well known that feeding growing animals or human diets containing raw *Phaseolus vulgaris* L. (PHV) as the sole or main source of protein causes a number of antinutritional effects (6). Several investigators have claimed that most if not all of the toxicity of PHV var. *processor* (PHVp) can be attributed to the specific action of the legume lectins (5, 9, 11). Lectins or phytohemagglutinins (PHA) are carbohydrate-binding and cell-agglutinating proteins and have long been widely used as mitogenic agents in immunology and cell biology (2). PHA are tetrameric glycoproteins composed of two different subunits, E and L, meaning erithroagglutinating and lymphoblastic activities, respectively, arranged into five different combinations (12). In recent papers (7, 8) it has been reported that feeding birds or mice raw legume diets caused an impairment in the immunological competence of the experimental animals. The aim of this work is to report recent data on the mitogenicity of the different isolectins isolated from PHV var. *athropurpurea* (PHVa), amply cultivated in Spain, on rat mesenteric lymphatic ganglia (MLG) lymphocytes.

From PHVa certified seeds, albumin and globulin fractions (AF, GF), as well

the isolectins E₄+E₃L, E₂L₂, EL₃ and L₄+lectin-free albumin were isolated, purified and identified by gel chromatography and SDS-PAGE (9). The amount of E₄ was negligible and remained attached to E₃L isolectin and with the procedure followed, L₄ isolectin could not be separated from the remainder lectin-free albumins. MLG lymphocytes were obtained from male Wistar rats (150-200 g b. wt.) as previously reported (4). See legend to Figure 1 for additional details. Cells were pulsed with [methyl-³H] thymidine and tritium labeled thymidine incorporation was assessed in a β -Beckman scintillation counter model LS 1800.

It has been found, first that PHVa isolectins are exclusively present in the AF, the GF being lectin-free; this contrasts with the results so far reported in studies carried out with PHVp and other varieties, where isolectins were homogeneously distributed between both AF and GF (5, 9, 10). Second, and in agreement with previously reported data, the highest mitogenic activity of PHVa isolectins was found after 72 h of activating MLG lymphocytes. And, finally (fig. 1), the mitogenicity of the different PHVa isolectin dilutions was found to be similar for all PHA tested, and therefore, it appears to

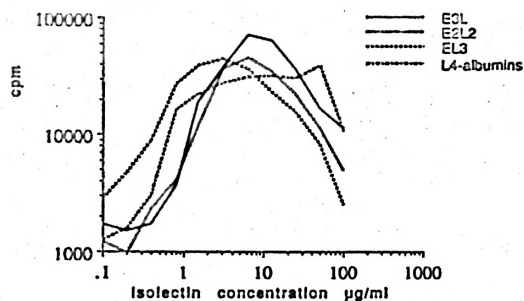


Fig. 1. Rat mesenteric lymphatic ganglia lymphocyte blastogenic proliferation responses to *Phaeo-scolus vulgaris* L. var. *athropurpurea* isolectins E_3+L , E_2L_2 , EL_3 and L_4 +lectin-free albumins. Lymphocytes were washed and adjusted to 2×10^6 cell/ml in a culture medium containing RPMI-1640, sodium bicarbonate 14 mM, HEPES 50 mM, heat inactivated fetal calf serum 5 %, L-glutamine, 50 µg/ml, 2-mercaptoethanol 5×10^{-5} M and antibiotics (100 µg gentamycin, 100 IU penicillin). In a multiwell plate, 200 µl of lymphocyte suspension were added in each well. The isolectins were added at progressive dilutions beginning with 200 µg/ml and incubated at 37 °C, 5 % CO_2 . One µCi (0.037 MBq)/well of tritiated thymidine was added and cells were harvested and counted for beta emission. The figure shows gaussian curves obtained after 3 days of incubating the cells, i.e. when the maximum response to all lectins occurred at the concentration of about 1.5-17.5 µg/ml. There were no mitogenic differences among the four isolectins tested.

be independent of their relative content of either the E or L subunits. These results correlate only in part with those of other investigators (1, 3) and are possibly related to the different affinity of PHVa isolectins with serum glycoproteins. Nevertheless, the outstanding similar mitogenicity of all PHVa isolectins on rat MLG lymphocytes has not as yet been reported and the immunological significance as well as the mechanisms underlying such an effect remain to be completely clarified.

Key words: Mitogenicity, Lymphocytes, Isolectins.

Palabras clave: Mitogenicidad, Linfocitos, Isolectinas.

References

1. Glad, C. and Borrebaeck, C. A.: *J. Immunol.*, 133, 2126-2132, 1984.
2. Goldstein, I. J., Hughes, R. C., Monsigni, M., Osawa, T. and Sharon, N.: *Nature*, 285, 66-68, 1980.
3. Hernández, D. E. and Leavitt, R. D.: *Cell Immunol.*, 86, 101-108, 1984.
4. Hudson, L. and Hay, F. C.: *Practical Immunology* (3rd ed.). Blackwell Scientific Publication, Oxford, 1989.
5. King, T. P., Pusztai, A. and Clarke, E. M. W.: *Histochem. J.*, 12, 201-208, 1980.
6. Liener, I. E.: *Toxic Constituents of Plant Foodstuff* (Liener, I. E., ed.). Academic Press, Inc. New York, 1982.
7. Macarulla, M. T., Marcos, R., Larralde, J. and Martínez, J. A.: *Brit. J. Nutr.*, 68, 493-503, 1992.
8. Marzo, F., Tosar, A. and Santidrián, S.: *Nutr. Rep. Intl.*, 46, 1189-1197, 1989.
9. Pusztai, A.: *Biochem. J.*, 94, 611-615, 1965.
10. Pusztai, A.: *Lectins*, vol. V. Walter de Gruyter and Co. New York, 1986.
11. Pusztai, A., Grant, G., Brown, D. S., Ewen, S. W. B. and Bardo, S.: *Med. Sci. Res.*, 16, 1283-1284, 1988.
12. Yachnin, S.: *Proc. Natl. Acad. Sci.*, 73, 2857-2865, 1976.

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