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Research Note

METHYL-α-GALACTOSIDE ENHANCES THE MITOGENICITY OF THE LECTIN JACALIN

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Jacalin, a galactose-binding protein, has a Mr of 50 kD, being composed of two noncovalently linked subunits of 12 and 15 kD (R. A. Moreira & I. L. Ainouz, 1981, *Biol. Plantarum, 23:* 186-192). Methyl-α-galactoside (1-0-methyl-α-D-galactopyranoside; Me-α-gal) is approximately 40 times more potent than galactose in inhibiting jacalin lectin activity (G. Suresh Kumar et al., 1982, *J. Biosc., 4:* 257-261; S. R. Dalmau & C. S. Freitas, 1989, *Brazilian J. Med. Biol. Res., 22:* 601-610). The present note shows that, instead, Me-α-gal improves the rat spleen mononuclear cell (RSC) proliferation when in overoptimal doses of the lectin.

In experiments carried out to test the relative potency of different sugar derivatives of blocking the jacalin-induced R strain RSC proliferation, we found that, when in overoptimal lectin doses, the addition of Me-α-gal increased the thymidine uptake above the levels obtained with optimal doses of jacalin. This unexpected effect could represent a shift in the dose-response curve, rather than a true enhancing effect on proliferation. We then used Simpson's rule (T. P. Sheeran et al., 1988, *J. Immunol. Methods, 115:* 95-98) in order to compare the areas under the proliferation curves obtained with different jacalin doses for up to 144 h of culture, in the presence or not of Me-α-gal. Since the SD of the means of the cpms did not exceed 7.5%, areas 15% (2xSD) higher or lower than that of the control curve were considered to be significantly different. As shown in Fig. 1, the largest area of RSC proliferation was found when using jacalin at an overoptimal dose (729 μg/ml) in the presence of 35 mM Me-α-gal.

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The enhancing capability of Me-α-gal can not be ascribed to a metabolic effect on cells, since at this concentration this sugar is somewhat inhibitory (18%) to IL-2-dependent RSC proliferation (Dalmau & Freitas, *loc. cit.*). To further exclude this possibility, we used rat erythrocyte ghosts as insoluble jacalin ligands in an attempt to reproduce the enhancement obtained with Me-α-gal. RSC were stimulated with various jacalin doses, in the absence or presence of 0.2 or 2% (v/v) of erythrocyte ghosts for 72 h. As shown in Fig. 2, both the maximal cpm and areas under curves were strongly increased by the addition of ghosts, specially at jacalin overoptimal doses (> 81 μg/ml). These results revealed that membrane-immobilized sugars with affinity for jacalin exert an effect on RSC proliferation similar to that of Me-α-gal, thus excluding a metabolic mechanism.

Me-α-gal has been reported to revert the suppression of cytotoxic T lymphocyte generation (M. A. Palladino et al., 1983, *J. Immunol., 130:* 2200-2202). We thus tested for the existence of a putative suppressor effect in the lymphocyte proliferative response which could be reverted by Me-α-gal. Fig. 3 shows the results obtained when RSC were stimulated with different doses of jacalin or concanavalin A (Con A), a mannose binding lectin, in the absence or presence of 35 mM of Me-α-gal or Me-α-man (1-0-methyl-α-D-mannopyranoside) for 72 h. No changes in the maximal cpm or areas were caused by Me-α-man or Me-α-gal on Con A-induced RSC proliferation. The increases, by Me-α-man, in the maximal cpm and area under the curve of the jacalin-induced RSC proliferation, somewhat lower than that with Me-α-gal, were not totally unexpected, since this sugar derivative was previously shown to bear an inhibitory potency on jacalin binding similar to that of racemic galactose (Dalmau & Freitas, *loc. cit.*).

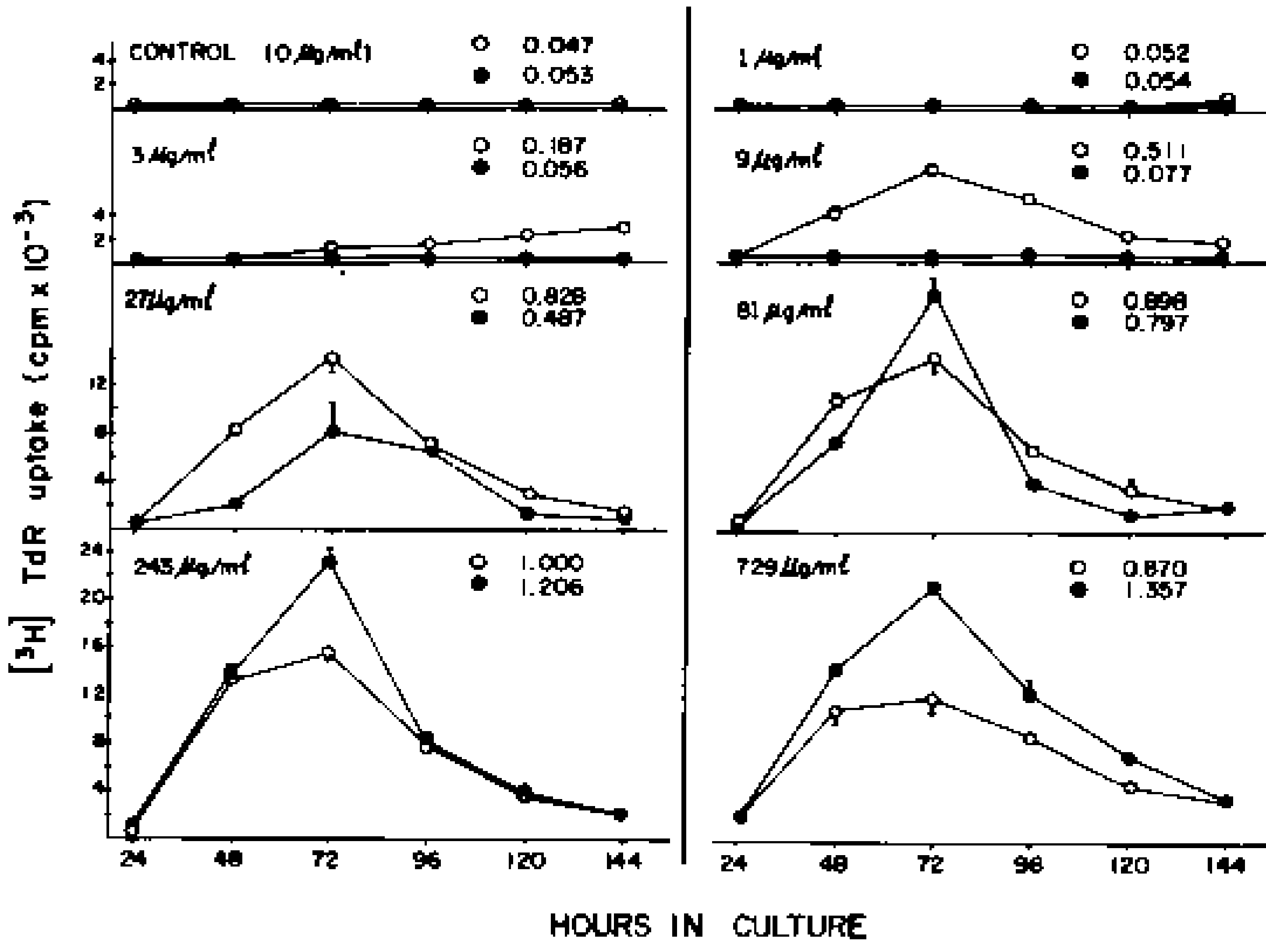

Fig. 1: the effect of Me-α-gal on jacalin-induced RSC proliferation. Erythrocyte-free RSC (R strain) were obtained by centrifuging spleen cell suspensions on a ficoll-hypaque solution (D = 1.088) for 30 min at 400 xG, and 18 °C. RSC were stimulated with the indicated doses of lectin and times of culture in the absence (O) or the presence (●) of 35 mM of Me-α-gal (Sigma Chem. Co., St. Louis, MO). Numbers beside symbols represent relative proliferative areas, taking as 1.00 the maximal proliferative area obtained in the absence of Me-α-gal. Cultures were run in triplicate with 1 x 10^5 RSC/0.2 ml/ well in RPMI 1640 medium (Sigma) plus 5% fetal calf serum (Cultilab, Campinas, SP) at 37 °C under a humidified 6% CO_2 in air mixture. Tritiated thymidine (0.5 µCi/ well, sp.act. 2 Ci/mmole, Dupont, Boston, MA) pulses lasted 6 h of each culture period. Vertical bars, SD.

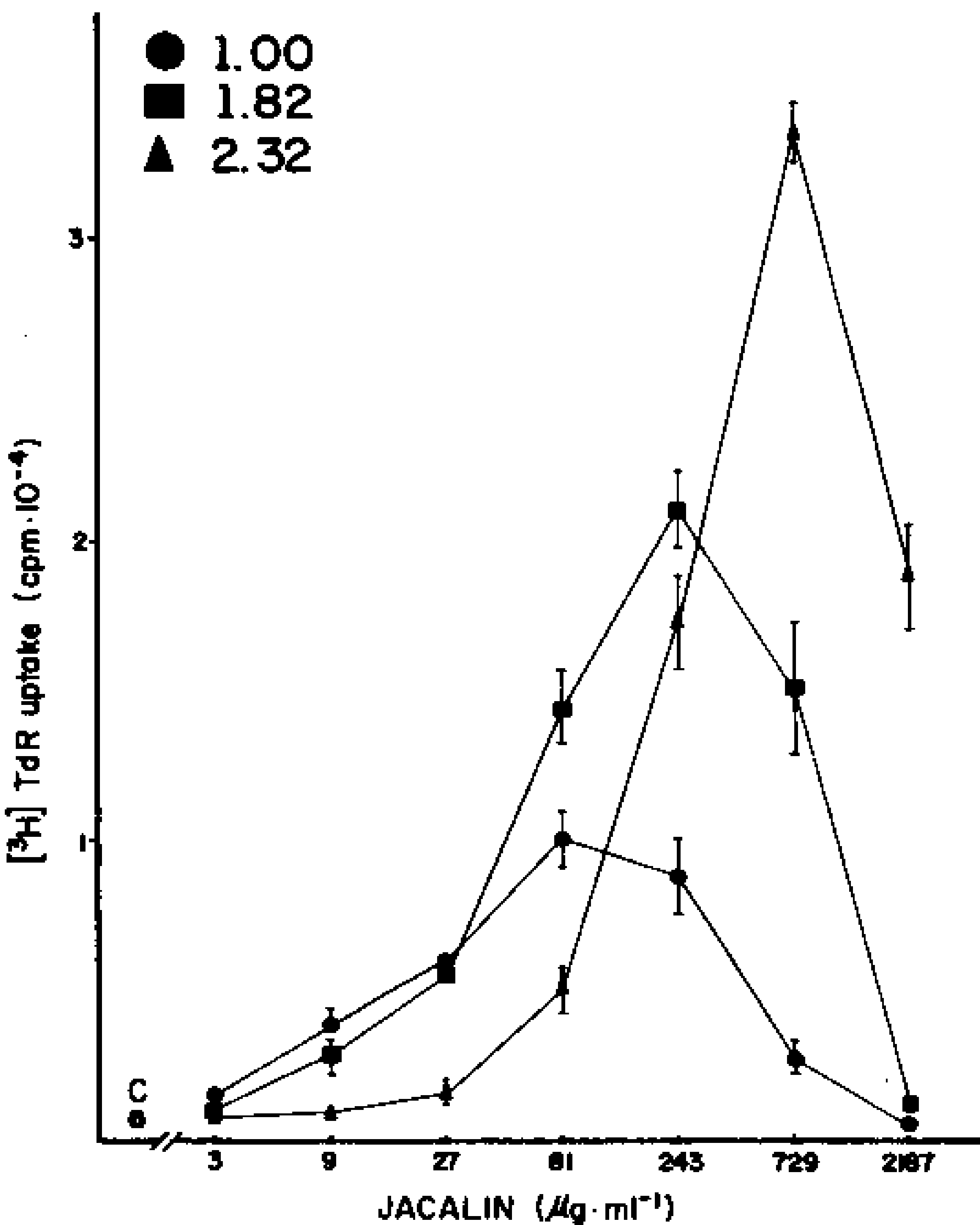

Fig. 2: the effect of erythrocyte ghosts on jacalin-induced RSC proliferation. Rat erythrocyte ghosts were prepared by three cycles of water lysis. The pelleted ghosts (3000 xG, 20 min) were suspended in culture medium for addition. RSC were stimulated with the indicated amounts of jacalin in the absence (●) or presence of 0.2 (■) or 2.0 (▲)% (v/v) of erythrocyte ghosts. Controls without jacalin, C. Cultures run as in Fig. 1, but for 72 h. Vertical bars, SD.

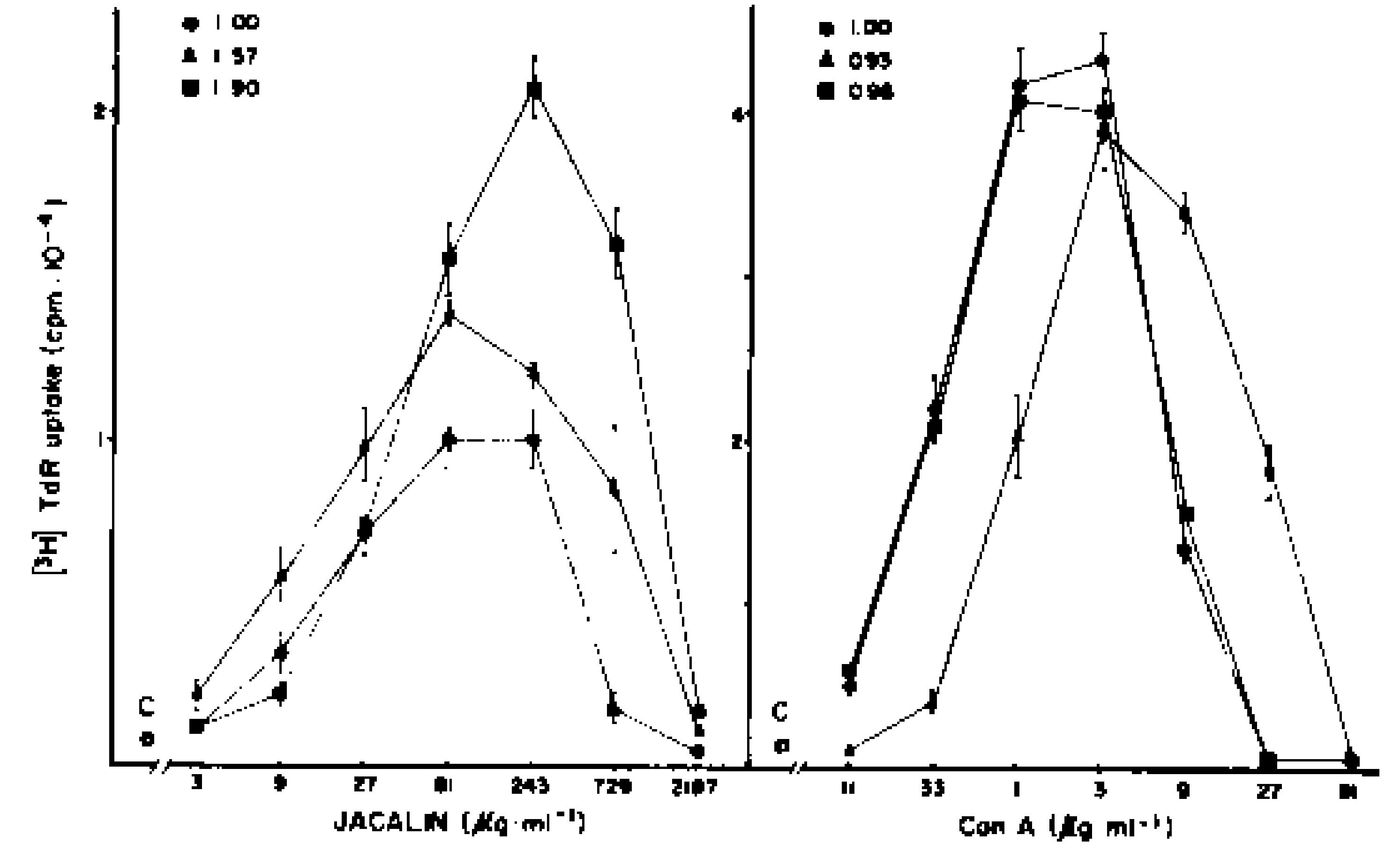

Fig. 3: the effect of Me-α-man or Me-α-gal on jacalin- and Con A-induced RSC proliferation. RSC were stimulated with the indicated amounts of jacalin (left panel) or Con A (right panel) in the absence (●) or presence of 35 mM Me-α-man (▲) or 35 mM Me-α-gal (■). Cultures without lectins, C. Cultures run as in Fig. 2. Vertical bars, SD.

These results indicate tha lack of a Me-α-gal-sensitive suppressor mechanism in the proliferative response to Con A, and show that, if it exists in the jacalin response, it must be specific for this lectin.

The mechanism by which Me-α-gal increases the overall mitogenic ability of jacalin at overoptimal doses is not clear. Our lectin preparation (S. R. Dalmau et al., 1989, *Brazilian J. Med. Biol. Res., 22:* 1111-1120), here referred as jacalin, shows three protein bands when analyzed by electrophoresis in agarose gels. In SDS-polyacrylamide gel eletrophoresis (SDS-PAGE) the main band (aprox. 60% of total protein) contains only the two polypeptides characteristic of affinity purified jacalin. Only one of the other two bands has mitogenic activity. On SDS-PAGE it is composed of at least five polypeptides with Mr greater than 25 kD. It has a similar specific activity, stimulates the RSC thymidine uptake to a same maximal level, and has the same pattern of sugar inhibition of the main band. However, it is much more toxic for RSC than the latter when in overoptimal doses (unpublished results). Thus, a hypothesis can be raised that Me-α-gal leads to an increased mitogenic stimulus by reducing the effect of a putative toxic moiety in our lectin preparation. The effect of Me-α-gal on RSC proliferation induced by overoptimal doses of these two bands remains to be assayed.

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