# LOSS OF MITOGENIC ACTIVITY BY IMMOBILIZED LECTINS

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SUMMARY: Concanavalin A and phytohemagglutinin P were immobilized on the bottom of Petri dishes and on agarose (Sepharose) and polyacrylamide (Bio-gel) beads. The various lectin derivatives obtained were tested for their capacity to stimulate DNA synthesis in human and mouse lymphocytes. In contrast to previous reports we found that stimulation of DNA synthesis in lymphocytes, as measured by the incorporation of tritiated thymidine was either absent or so slight as to be of questionable significance.

INTRODUCTION: It is now well established that the water soluble plant lectins phytohemagglutinin from *Phaseolus Vulgaris* (PHA)[1] and *Concanavalin A* (Con A) from *Canavalia Ensiformis* are capable of stimulating DNA synthesis in thymus derived (T lymphocytes), although not in bone-marrow derived lymphocytes (B lymphocytes) of mice. Recently several reports have appeared suggesting that these lectins, after their immobilization onto water insoluble supports, are no longer capable of stimulating DNA synthesis in T lymphocytes but are able to do so in B lymphocytes (1-3). The finding would be obviously important since it would help to analyse the mechanism of lymphocyte activation by antigens.

In our work, centering on the stimulation of lymphocytes by insolubilized antigens, we chose the lymphocyte-insoluble lectin system as a model. We repeatedly attempted to stimulate lymphocytes with insoluble plant lectins but were unsuccessful. In the present paper we describe the experiments which we performed. We found that DNA synthesis in lymphocytes cultivated in the

[1]The following abbreviations have been used : PHA, phytohemagglutinin ; Con A, Concanavalin A ; $H^3$-TdR, tritiated thymidine.

presence of insoluble lectins, as measured by the incorporation of tritiated thymidine ($H^3$-TdR), was so slight as to be of questionable significance.

MATERIALS AND METHODS: Con A was isolated from Jack bean meal according to the procedure of Agrawal and Goldstein (4). Purified phytohemagglutinin P was obtained from Wellcome Research Laboratories (Beckenham UK). These lectins were bound to cyanogen bromide activated agarose gel beads (Sepharose) following exactly the methods described by Andersson and Melchers (2) and Greaves and Bauminger (3). By these procedures 3 mg of Con A and 1 mg of PHA per ml of packed Sepharose beads were fixed. Con A and PHA were also immobilized on glutaraldehyde activated polyacrylamide beads (Bio-gel P-300) following a procedure already described (5). By this procedure 1.5 mg of Con A and 1 mg of PHA per ml of packed Bio-gel beads were fixed.

The bottom of a series of Petri dishes (30 mm) was coated with Con A according to a procedure already described (6). The quantities of lectin bound to the dish were calculated from parallel experiments using $^{125}I$-labelled lectins. E. coli lipopolysaccharide (LPS) was kindly donated by Dr. C. Bonna (Institut Pasteur).

Normal human lymphocytes were collected with an IBM cell separator. Mouse lymphocytes were obtained from spleens of normal DBA/2 and C57 Bl/6 animals and from mouse homozygotes for the nude character (nu/nu), kindly supplied by Dr. J.C. Salomon (CNRS Institut, Villejuif). The spleens were removed aseptically and teased gently in MEM medium. The cells were then passed through a sterile nylon mesh column, washed three times by centrifugation and finally suspended in the nutrient medium at a concentration of 1 or $5x10^6$ cells/ml. The incubation was done in the presence or not of various quantities of insoluble lectins at 37°C in an atmosphere of 5% $CO_2$ and 80% humidity. The nutrient medium used was either MEM or RPMI 1640 supplemented with 5 or 10% human or foetal calf serum, decomplemented or not. Three types of culture vessels were used : Petri dishes, glass tubes and microplates. On the 2nd or 3rd day after starting the culture, 1 µCi of $H^3$-TdR per ml of culture was added. After 18 hours, the cells were washed twice with cold isotonic saline, hydrolyzed with 2 ml of 1N OHNa and precipitated with 2 ml of 30% perchloric acid. The precipitate was washed twice with 5% perchloric acid and dissolved in 10 ml of Instagel (Packard Co). The radioactivity of this solution was measured in a liquid scintillator counter.

RESULTS AND DISCUSSION: Since bacteria are producing substances which even in minute amounts can stimulate proliferation of B lymphocytes (7), much care was taken during the present work in order to avoid bacterial contamination. Maximum possible sterile conditions were employed during all working steps in the preparation and use of the insoluble lectin derivatives.

Two different experiments were carried out with cells incu-

TABLE 1

C.P.M.* per culture found in human lymphocyte after 3 days incubation in Petri dishes coated or not with Concanavalin A

| Dishes uncoated | Dishes uncoated | Dishes coated | Dishes coated with Concanavalin A (µg per dish) | | | | |
|---|---|---|---|---|---|---|---|
| | 10 µg of soluble Concanavalin A added per ml of medium | with bovine serum albumin | 150 | 75 | 38 | 19 | 10 |
| 2,888 | 97,118 | 2,054 | 2,119 | 1,853 | 2,442 | 2,204 | 2,016 |

* Counts per minute ; mean of four samples.

TABLE 2.

C.P.M.* per culture found in spleen lymphocytes from nude mice 3 days after incubation with or without Concanavalin A immobilized on Sepharose beads

| Without beads | Without beads | Without beads | Immobilized Concanavalin A added per ml of medium (in µgs) | | | | Immobilized Bovine serum albumin added per ml of medium (in µgs) | |
|---|---|---|---|---|---|---|---|---|
| | 150 µg of lipopolysaccharide added per ml of medium | 10 µg/ml Concanavalin A added per ml of medium | 100 | 10 | 1 | 0.1 | 100 | 10 |
| 4076 | 183565 | 5795 | 27588 | 10289 | 9181 | 6480 | 31764 | 8224 |

* Counts per minute ; mean of three samples.

bated with Con A bound to Petri dishes and 9 with PHA. The results obtained were similar in all these experiments. A typical result of such an experiment is shown in Table 1. In this experiment human lymphocytes were incubated in dishes carrying various densities of Con A. It is evident that the insoluble Con A did not in any of the concentrations tested increase the incorporation of $H^3$-TdR. On the other hand, soluble Con A added at 10 μg/ml in the culture medium stimulated significantly the incorporation of $H^3$-TdR by the lymphocytes.

Seventeen different experiments were carried out using various preparations of mitogens (13 with Con A and 4 with PHA) bound to Sepharose (10 experiments) and Bio-gel beads (7 experiments). In Table 2 a representative experiment, using Sepharose beads coated with Con A and lymphocytes from nude mice, is given. It can be seen that the soluble lipopolysaccharide from E. coli stimulates significantly the incorporation of $H^3$-TdR in lymphocytes. Under the same conditions 10 μg/ml of soluble Con A was without any significant effect. Since lipopolysaccharide is a B cell mitogen while Con A is a T cell mitogen (7) and since in nude mice mostly B cells are present (8), the results obtained are those expected. From the results presented in Table 2 it can also be seen that insoluble Con A at high concentration (100 μg/ml) seems to increase the incorporation of $H^3$-TdR by the cells, although not to the same extend as in the cultures containing LPS. However the same increase in the incorporation of $H^3$-TdR is noted in the control experiments where the cells were incubated with the same dilution of Sepharose beads coated with BSA. In fact in several experiments we found that $H^3$-TdR can bind directly to the beads. Since the beads, at high concentrations can appear in the same fraction with the cells, this can give a fallacious impression of cell bound radioactivity when proper controls are not used. In only a few experiments (3 out of 17), in comparison with the control preparations, we found a maximum of 3 times higher incorporation of $H^3$-TdR in cells incubated with insoluble Con A. Since this increase was observed only with the highest concentration of insoluble Con A the significance of this stimulation effect seems questionable. It is quite possible that at these high concentrations a few micrograms of Con A leaked out from the beads into

the incubation medium. The quantity would be sufficient to stimulate some T cells (which are known to be present, although in very low percentage, even in lymphocytes taken from nude mice) (8) to produce and secrete B cells mitogen factors. Alternatively, these low amounts of solubilized Con A could be present in such a form as to be able to directly stimulate B cells. Finally, stimulation could also be due to the presence of B cells mitogens produced during a bacterial contamination.

In conclusion, therefore, we cannot confirm the previous reports suggesting that insoluble lectins can activate directly B lymphocytes.

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