

Technical note

Effect of different lectins on the proliferation of goat peripheral blood mononuclear cells

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Abstract

In the present study we determined the effect of the lectins concanavalin A (Con A), phytohemagglutinin (PHA) and pokeweed (PWM), frequently used in cell proliferation assays, on the proliferation of goat peripheral blood mononuclear cells (PBMCs). The use of a standardized Percoll gradient proved to be highly effective for the separation/enrichment of these cells, without damaging the cell membrane. Pokeweed and phytohemagglutinin showed a high stimulating effect, with proliferation rates of 16,753 and 2350 counts/min (cpm), respectively, whereas Con A showed very low stimulation indices (495 cpm), supporting the notion that it should not be used for blast proliferation studies in goats.

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1. Introduction

The stimulating effect of plant-derived mitogens (lectins) on various cell subpopulations has been well described for some animal species (Arunachalam et al., 1989; Moreira et al., 1991). However, few reports are available about the ideal concentrations needed to reach good proliferation rates in goat peripheral blood mononuclear cells (PBMCs) in vitro. The determination of these concentrations for lectins is of fundamental importance since they are the tools which are extensively used in studies of basic cell processes and in assays for the evaluation of the cellular

immune response. While there are other methods to evaluate stimulatory or inhibitory antigen activity, the use of lectins is justified by their low price and because of the low availability of goat specific reagents. The objective of the present study was to determine the lectin concentrations that would permit their use as controls in experiments conducted to evaluate the cellular response of goats (Fig. 1).

2. Materials and methods*2.1. Animals*

The study was conducted on 10 apparently healthy male and female goats of undefined breed aged 6–8 months, kept in Camaçari, Bahia, Brazil.

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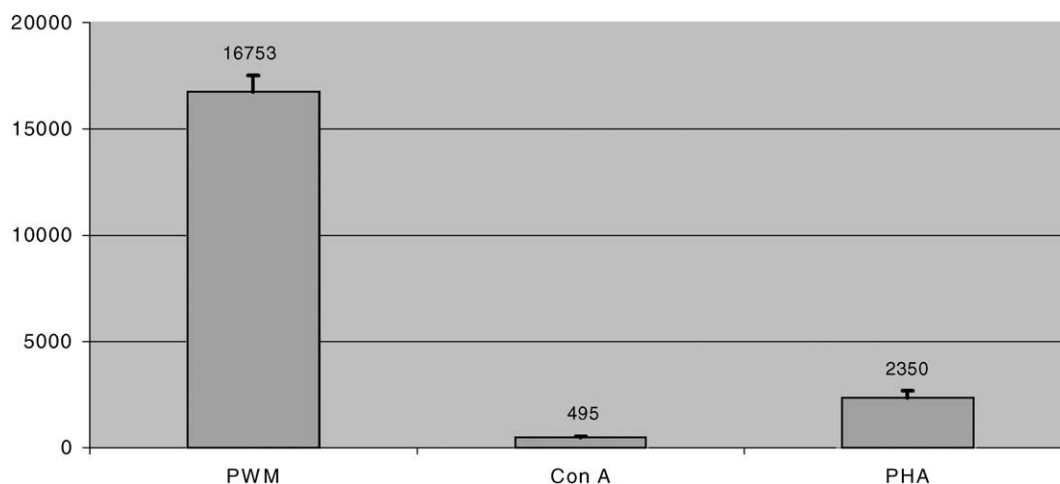


Fig. 1. Effect of different lectins on goat PBMC. Cells (4×10^5) were incubated with PWM, PHA and Con A for 72 h. Results are expressed as counts/min.

2.2. Culture medium

The culture medium used was RPMI-1640 (Gibco BRL) supplemented with 2 mM L-glutamine, 25 mM HEPES, 10% fetal calf serum, and 1% PSN antibiotic mixture (Gibco), pH 7.4.

2.3. Mitogens

The mitogens used were concanavalin A (Con A), pokeweed (PWM) and phytohemagglutinin (PHA), all from Sigma. An initial concentrated solution of 1 mg/ml phosphate buffered saline (PBS) was prepared for all mitogens and serial dilutions were later prepared up to the final concentration to be added to the wells of cell culture plates.

2.4. PBMC separation on a density gradient (Percoll)

Blood was collected with EDTA by jugular vein puncture, diluted 1:3 in PBS, and applied to the Percoll layer (previously adjusted to a density of 1.077) in a 15-ml Falcon tube (Corning). The material was centrifuged at $2000 \times g$ for 50 min and the interface ring containing PBMC was collected, washed three times in PBS and resuspended in 1 ml RPMI for counts. The integrity of the cell membrane as determined by Trypan Blue exclusion was more than 97%. The cell concentration was adjusted to 5×10^6 cells/ml.

2.5. Culture preparation for cell proliferation assays

PBMC of each individual were distributed in 96-well cell culture plates (Coastar) at the concentration of 4×10^5 cells per well. The mitogens were used at the following concentrations: PWM, 1 μ g per well; Con A, 5 μ g per well; and PHA, 5 μ g per well (Kruisbeek and Sheevach, 1991). After 72 h of incubation, the cells were pulsed with tritiated thymidine (1 μ Ci per well), and collected on fiberglass paper (Packard). The β emission was determined with an automatic counter.

2.6. Statistical analysis

The mitogen effect over the lymphoproliferation was tested by Repeated Measures Analysis of Variance and the measures were compared by the Turkey–Kramer multiple comparisons test. The variability of the data distribution were tested by the Kolmogorov and Smirnov analysis.

3. Results and discussion

Best separation occurred when 4 ml diluted blood was applied to the same volume (4 ml) of Percoll (Pharmacia). Lymphocyte purification or enrichment by the Percoll gradient at 1.077 density was

Table 1
Proliferation rates after PBMC stimulus with lectins for 72 h

Animals	PWM	Con A	PHA
1	16,556	550	2124
2	17,102	504	2850
3	17,324	445	2210
4	16,900	490	2812
5	15,879	400	2247
6	17,960	560	2189
7	15,753	445	2532
8	17,652	425	2588
9	16,504	574	1987
10	15,900	557	1961
Mean (S.D.)	16,753 ^a (769)	495 ^c (64)	2350 ^b (324)

Results are expressed as counts/min. Means with different letters (a, b and c) are statistically different ($P < 0.001$).

satisfactory. Although there are reports of the use of Ficoll–Paque as a density gradient for goats (Roth et al., 1991; Ram et al., 1987), we opted for the use of a Percoll gradient because of the possibility of adjusting density.

The integrity of the purified/enriched cells, determined by Trypan Blue exclusion, was more than 97%, a value similar to that reported by Tomar et al. (1993).

Under the conditions used, PWM at the concentration of 1 µg per well was the mitogen showing the greatest stimulation of goat PBMC proliferation, with a mean proliferation index of 16,753 counts/min (cpm) in triplicate experiments. PHA at the concentration of 5 µg per well showed a proliferation index of 2350 cpm, and Con A at the concentration of 5 µg per well showed mean values of 495 cpm. Ram et al. (1987) also reported a low efficiency of Con A in stimulating goat PBMC (Table 1).

4. Conclusions

- The Percoll gradient standardized in the present study, which has not been previously reported in the literature for goats, well suited for the separation/enrichment of peripheral blood cells of this species.
- The integrity of the membrane suggests that Percoll has no toxic action, although staining with other dyes has been indicated, such as acridine orange and ethidium bromide, which permit the observation of cell viability.
- At the concentrations and under the conditions used, blastogenic induction was better observed with PWM and PHA, with proliferation rates of 16,753 and 2350 cpm, respectively. Con A showed very low stimulation indices (495 cpm), supporting the recommendation that it should not be used for blast proliferation studies in goats, as previously indicated by Ram et al. (1987).

References

- Arunachalam, B., Subba Rao, M.V., Ram, G.C., 1989. Bovine interleukin-2: biochemical and biological characterization. *Vet. Immunol. Immunopathol.* 23, 377–383.
- Kruisbeek, A.M., Sheevach, E., 1991. Proliferative assays for T cell function. *Curr. Protocols Immunol.*, John Wiley and Sons, 3.12.1.
- Moreira, R.A., Ainouz, I.L., Oliveira, J.T.A., Cavada, B.S., 1991. Plant lectins, chemical and biological aspects. *Mem. Instituto Oswaldo Cruz* 86, 211–218.
- Ram, G.C., Subba Rao, M.V., Singh, V.P., Bansal, M.P., 1987. Isolation and characterization of goat interleukin-2. *Indian J. Exp. Biol.* 25, 589–591.
- Roth, T.L., White, K.L., Horov, D.W., 1991. Suppression of sheep and goat proliferation by sheep. *J. Anim. Sci.* 69, 4563–4569.
- Tomar, A., Bansal, M.P., Ram, G.C., 1993. Effect of co-stimulants on interleukin-2-like activities from goat peripheral blood mononuclear cells. *Vet. Immunol. Immunopathol.* 38, 367–373.