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Brief Communication

LYMPHOCYTE STIMULATION AND LEUCOCYTE MIGRATION INHIBITION AS DIAGNOSTIC TOOLS FOR THE DETECTION OF MYCOBACTERIUM AVIUM INFECTION IN CATTLE

The tuberculin skin test is the conventional method of detecting infections with mycobacteria in animals. A positive reaction is considered to reflect cell-mediated immunity (CMI). CMI against mycobacteria can be studied by in vitro systems using suspensions of blood lymphocytes or leucocytes. The reactivity of these cells to different antigens can be measured in the lymphocyte stimulation (LS) (*Muscoplat et al.* 1975, *Bergman* 1976, *Johnson & Morein* 1976), or leucocyte migration inhibition (LMI) (*Aalund* 1970, *Clausen* 1973) tests.

Infections with atypical mycobacteria occur with varying frequency in cattle (*Schliesser* 1976). These infections sometimes interfere with the tuberculin skin tests against typical mycobacteria and M. paratuberculosis, giving so-called false reactions. For this reason test systems are desirable which can discriminate between infections with different species of mycobacteria.

The object of the present work was to adapt and evaluate two in vitro tests, namely lymphocyte stimulation and leucocyte migration inhibition in their ability for differentiation of mycobacteral infections in cattle.

Two 12-month old Ayrshire cows were used in the experiments. After experimental immunization with live M. avium the cows were positive in skin tests four weeks later with protein purified derivative (PPD) from this bacterial species.

The LS and LMI tests were carried out four times during the following 30-day period. Intradermal tuberculin tests were made simultaneously with the in vivo tests. The LS test was used according to the method of *Junge et al.* (1970). The stimulation index (SI) was calculated by the formula

$$SI = \frac{\text{mean CPM of cultures stimulated with PPD}}{\text{mean CPM of replicate control cultures}}$$

in which CPM means counts per minute of ^{3}H-thymidine.

The leucocyte migration inhibition test was performed essentially according to the technique described by *Clausen* and adapted to bovine blood cells by *Moreno-López* (in press). The degree of cell migration was calculated for each well.

PPDs* from bovis, M. avium or M. intracellulare were used to stimulate the lymphocyte and leucocyte cultures. In preliminary experiments 1 µg PPD was an optimum dose for the LS test. In the LMI test 10 µg or 50 µg gave equal results, and in the skin test 10 µg of each PPD was used.

Table 1. Response to homologous and heterologous PPDs following sensitation of two cows with Mycobacterium avium.

Animal	PPD antigen[a]	Lymphocyte stimulation index (see text)[b]	Leucocyte migration inhibition in %[b]	Skin fold thickness increase in mm[b]
Cow 1	M. avium	6.5	32.0	6.0
	M. intracellulare	1.4	8.5	4.0
	M. bovis	1.1	0	0
Cow 2	M. avium	5.0	27.5	6.0
	M. intracellulare	1.3	5.5	4.0
	M. bovis	1.2	0	0

[a] 1 µg was used in the LS test, 10 µg in the LMI test, and 10 µg in the skin test.

[b] Mean values from four experiments during a test period of one month.

The results obtained with the LS, LMI, and skin tests are presented in Table 1. In the LS test the homologous PPD caused a SI of 5.0 and 6.0 in the two cows. PPD from M. intracellulare and M. bovis gave SI values of 1.4 and 1.1 in one cow and of 1.3 and 1.2 in the other one.

Inhibitions of 27.5 % and 32.0 %, respectively, were found with homologous PPD in the LMI test, whereas PPD from M. intracellulare caused inhibitions of 5.0 % and 8.5 %, respectively. No inhibition of leucocytes was observed with the PPD from M.

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bovis. Skin fold thickness increase was 6.0 mm for homologous PPD and 4.0 mm for the PPD of M. intracellulare. No reaction in the skin could be detected with the PPD of M. bovis.

These results indicate that cows sensitized to M. avium could be recognized in both cell tests by means of PPD antigens. It seems obvious that these in vitro tests are more specific than the tuberculin skin test, and that mycobacterial antigens responsible for cell-mediated immunity might be used for detection of infections with closely related mycobacteria.

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REFERENCES

Aalund, O.: The migration test on circulating bovine leukocytes and its possible application in the diagnosis of Johne's disease. Acta vet. scand. 1970, *11*, 331—334.

Bergman, R.: Cell-mediated immunity in pigs infected with Mycobacterium avium. Zbl. Bakt., I. Abt. Orig. 1976, A *35*, 274.

Clausen, J. E.: Leucocyte migration agarose test. Acta allerg. (Kbh.) 1973, *28*, 172—179.

Johnson, K. & B. Morein: In vitro stimulation of bovine circulating lymphocytes by Parainfluenza Type 3 virus. Res. Vet. Sci. 1976. In press.

Junge, V., J. Hoekstra, L. Wolfe & F. Deinhardt: Microtechnique for quantitative evaluation of in vitro lymphocyte transformation. Clin. expt. Immunol. 1970, 7, 431.

Moreno-López, J.: Cellmediated immunity to parainfluenza-3 (PIV-3) virus in cattle. Evaluation of in vivo and in vitro tests. Zbl. Vet.-Med. B. In press.

Muscoplat, C., C. Thoen, A. Chen, P. Rakich & D. Johnson: Development of specific lymphocyte immunostimulation and tuberculin skin reactivity in swine infected with Mycobacterium bovis and Mycobacterium avium. Amer. J. vet. Res. 1975, *36*, 1167—1171.

Schliesser, Th.: Vorkommen und Bedeutung von Mykobakterien bei Tieren. (Occurrence and importance of mycobacteria in animals). Zbl. Bakt. I. Abt. Orig. 1976, A *35*, 274.

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