EVALUATION OF CELL-MEDIATED IMMUNE RESPONSE FOR SWINE DYSENTERY

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Recently, several in vitro methods were described which measured the inhibition of migration of various indicator cells as an assay of cell-mediated immunity (CMI) response in swine infected with different diseases. The leukocyte migration-inhibition agarose test (LMAT) was carried out to detect CMI response in swine infected with African Swine Fever virus, pseudorabies virus, and transmissable gastroenteritis virus (Shimizu et al., 1976; Gutekunst, 1979a; Wood, 1977). More recently, we demonstrated CMI responses in swine dysentery (SD) (Jenkins, 1978; Jenkins and Klesius, 1980). The purpose of this study was to evaluate and compare the in vitro LMAT with delayed type hypersensitivity (DTH) for the diagnosis of SD.

Swine experimentally infected with Treponema hyodysenteriae and uninfected ones were used in the experiments. Twenty-five ml heparinized blood was collected from each animal from the cranial vena cava. The peripheral blood leukocytes (PBL) were collected from the blood in the same syringe on 5 ml of 6% solution of Dextran (mol. wt. 282,000) in 0.9% saline solution after the RBC were allowed to settle 30 minutes at room temperature. Then with the same needle bent at 45 degrees, the PBLs were transferred to 15 ml sterile polystyrene test tubes and centrifuged at 2,000 rpm for 30 minutes. After centrifugation, the plasma was discarded and the precipitant was treated with 5 ml buffered NH_4Cl for 5 minutes to lyse the RBC. An equal volume of citrate saline solution was then added to restore isotonicity and the cells were spun at 2,000 rpm for 10 minutes. The cells were then washed 2x in liquid culture medium and counted using the Hycel Counter (American Scientific Company, Atlanta, Georgia). The suspension was adjusted to contain 4×10^6 cells/well and centrifuged at 2,000 rpm for 5 minutes. The supernatants were then aspirated and the cells resuspended in 40 µl of liquid culture medium with or without antigen as dictated by experimental protocol. Four 10 µl portions were then added to each of four wells in the agarose medium. A 1% solution of agarose dissolved in Earl's Balanced Salt Solution (containing 15% fetal calf serum and 100 IU/ml of penicillin) adjusted to pH 7.4 with $NaHCO_3$ was used. These plates were incubated from 9-24 hours at 39°C, with 5% CO_2 tension in a water vapor saturated environment. The other method of incubation was by using CO_2 GasPak (Baltimore Biological Laboratory, Cockeysville, MD) and incubating at 37°C from 24-48 hours.

After incubation, the plates were fixed with 3% glutaraldehyde for 30 minutes and the agarose removed. The degree of inhibition was calculated as migration index (MI) or as percent inhibition as previously described (Jenkins, 1979).

In the experiments carried out to demonstrate the effect of timing of LMAT with the use of CO_2 incubator and GasPak, there was a significant MI ($P < 0.05$) after 9 hours with an optimal incubation period of 12 hours under the CO_2 incubation. In contrast, under the GasPak, an optimal 24 hours was required for inhibition.

For the DTH reaction swine of different stages of infection were injected intradermally with 0.3 ml of T. hyodysenteriae antigen, trechalonacetic acid extract (TCAE) on the pinnas of the ear. The reactions were read at 6, 24, 48 and 72 hours, and a skin reaction of $\geq$ 5 mm than that of the saline control was considered positive.

A comparison of the LMAT and DTH during the course of SD indicated that the highest number of reaction was observed during the convalescent stage of the disease (Table 1) for both the LMAT and the DTH (86.21% and 60.72%, respectively, of the animals reacted). During the acute stages of the disease, 60.34% of the animals responded to the LMAT while 51.72% reacted to the DTH. The same pattern was true for the chronic state of the disease where 64.40% of the animals reacted to the LMAT and 50% responded to DTH.

Table 1. Comparison of leukocyte migration-inhibition agarose test and delayed type hypersensitivity.

LMAT		
No. of Animals Tested	Course of Disease	No. and % of Positive Reactors
57	pre-exposed	6 (10.52)
58	acute	35 (60.34)
58	convalescent	50 (86.21)
59	chronic*	38 (64.40)

DTH		
No. of Animals Tested	Course of Disease	No. and % of Positive Reactors
27	pre-exposed	4 (14.81)
29	acute	15 (51.72)
28	convalescent	17 (60.72)
24	chronic	12 (50.00)

*4-6 months post-exposure

Biopsies of skin test sites at 24 and 48 hours in sensitized pigs showed a perivascular, deep dermal inflammatory response consisting largely of lymphocytes and neutrophils. A less prominent infiltrate of similar composition appeared in the more superficial layers of the dermis. Control animals had no inflammatory response (although there was an increase at the surface of the skin due to injections of saline which disappeared later).

Conclusion:

Although the results indicated that the DTH may well by useful as an in vivo test to correlate CMI response, the LMAT appears to be more efficient in the detection of SD-affected animals. The superiority of the LMAT was due to the following: (1) The ease of obtaining target cell population; (2) The rapidity of the method; and (3) Lack of requirement of expensive materials and equipment.

Selected references: Shimizu, M., Pan, I.C., Hess, W.R.: Am. J. Vet. Res. 1977, 38:27-31; Woods, R.D.: Am. J. Vet. Res. 1977, 38:1267-1269; Gutekunst, D.E.: Am. J. Vet. Res. 1979, 40:66-68; Bendixen, P.H.: Am. J. Vet. Res. 1977, 38:1161-1162; Jenkins, E.M.: Am. J. Vet. Res. 1979, 41:338-340; Jenkins, E.M. and Klesius, P. L.: Vet. Imm. & Immunopath. 1981, 2: 19-26.

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