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# A blastogenic test for foot-and-mouth disease

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SUMMARY

A blastogenic test to detect peripheral blood leukocytes specifically sensitized to foot-and-mouth disease virus antigen is described. The test is carried out in microtitre plates and optimum conditions were found by titration. These employed $7{\cdot}5 \times 10^5$ cells/well and 20 complement fixing units of antigen. Peak [$^3$H]thymidine incorporation was found to take place at 2–3 days.

## INTRODUCTION

Both the humoral and cellular limbs of the immune system are involved in recovery from virus infection (Allison, 1972, 1974) and although either component may act decisively in eliminating virus (Babiuk, Wardley & Rouse, 1975; Rouse & Babiuk, 1975) it is probable that there is a complex interaction between the two. With foot-and-mouth disease virus (FMDV), where there is a good correlation between antibody levels and protection in cattle (Martin & Chapman, 1961; van Bekkum, 1969), cell-mediated reactions have not been investigated in depth and it would appear worth while to apply more recently developed techniques to determine if a cell-mediated function is of importance in this disease.

## MATERIALS AND METHODS

### *Animals*

Adult Devon steers were used throughout the experiment. Previously these animals had been inoculated with two doses of inactivated type O FMDV vaccine (a gift from the Wellcome Foot-and-Mouth Disease Vaccine Laboratory, Pirbright Surrey). On entering the experiment, a third dose of vaccine was given.

### *Peripheral blood leukocytes*

Heparinized venous blood taken from the Devon steers was used for the isolation of PBL by Ficoll-Hypaque flotation (Wardley, 1977). These cells were then suspended in HEPES (20 mM) buffered RPMI 1640 medium containing 10% heat-inactivated (56 °C, 30 min) fetal calf serum (RPMI-HEPES).

### *Antigen*

Antigen used in the blastogenic test was prepared by growing FMDV (type O-BFS 1860) in either BHK or IB-RS-2 monolayers and then purifying the 140S

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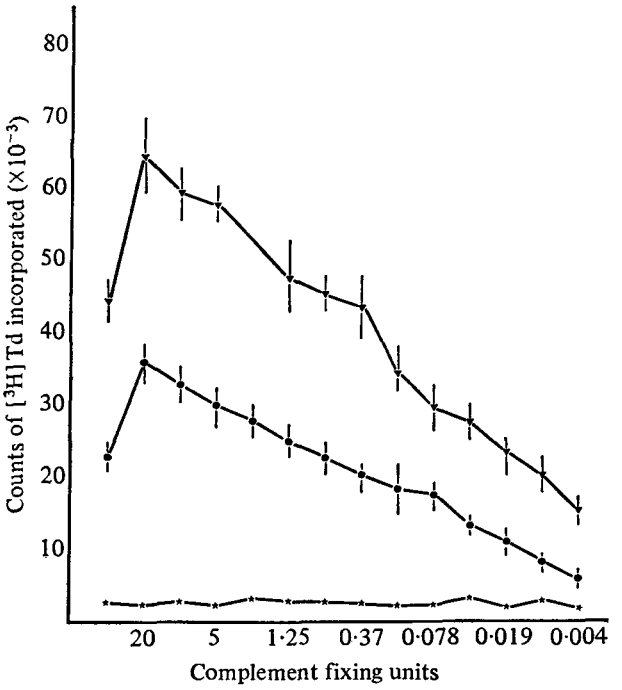


Fig. 1. Effect of antigen concentration on [$^3$H]thymidine incorporation. ▼—▼ and ●—●, Vaccinated animals; ◆—◆, control animal.

component by sucrose density centrifugation (Brown & Cartwright, 1963). Virus was then inactivated using AEI (Brown & Crick, 1959) and dialysed against phosphate buffered saline (PBS) before storage at −70 °C.

*The blastogenic assay*

All assays were carried out in round-bottomed microtitre plates (Cooke Microtitre Systems, U.K.). PBL were diluted in RPMI-HEPES and 200 $\mu$l volumes containing known numbers of cells were dispensed into the plates. Twenty $\mu$l amounts of antigen were then added to some wells and the plates incubated in a humidified incubator (5% $CO_2$, 95% air) at 37 °C for various times before the addition of 1 $\mu$Ci of [$^3$H]thymidine (The Radiochemical Centre, Amersham, U.K.) to each well. After a further incubation period of 18 h, plates were harvested using a PAM harvester (Ilacon, U.K.) and the incorporation of [$^3$H]thymidine assayed using a scintillation counter. Results were expressed as counts from wells receiving antigen minus counts from wells without antigen. PBL from non-vaccinated cows were used as cell controls and uninfected cell supernatants and the various reagents used during the processing of the antigen as antigen controls.

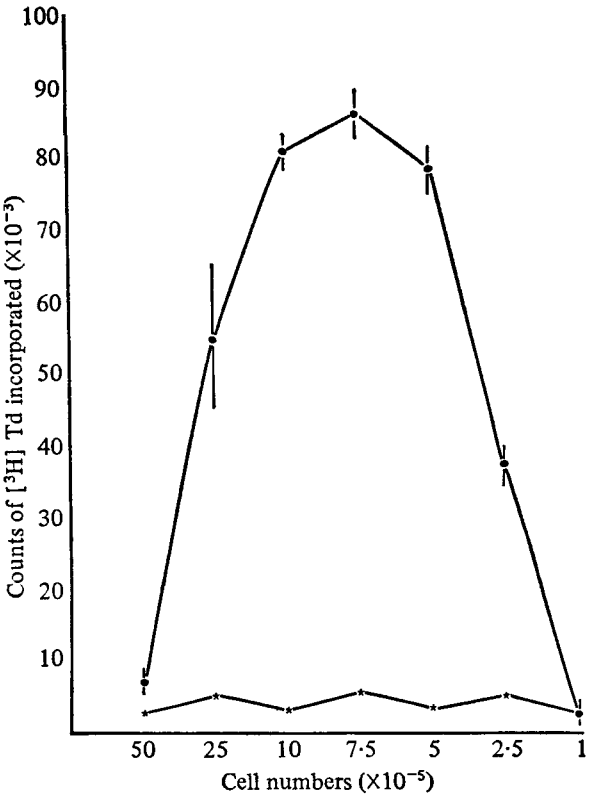


Fig. 2. Effect of cell concentration on [$^3$H]thymidine incorporation. ●—●, Vaccinated animal; ◆—◆, control animal. Each point represents mean of six determinations, bar 1 S.D.

## RESULTS

### *Effect of antigen concentration on [$^3$H]thymidine incorporation*

Typical results showing titrations of antigen are given in Fig. 1. The peak response occurred at 20 complement-fixing units. Concentrations of antigen in excess of 20 complement-fixing units/well had an inhibitory effect.

### *Effect of cell concentration on [$^3$H]thymidine incorporation*

Results of these experiments are shown in Fig. 2. Optimal cell concentrations were between $1 \times 10^6$ and $5 \times 10^5$ PBL/well and $7{\cdot}5 \times 10^5$ cells/well was the concentration chosen for use in future experiments.

### *Effect of pulse time on [$^3$H]thymidine incorporation*

Plates were set up at $7{\cdot}5 \times 10^5$ PBL and 20 complement-fixing units per well, and [$^3$H]thymidine was added at different times over a period of 7 days. The results of this experiment, shown in Fig. 3, indicate that peak incorporation occurred between 2 and 3 days, and 3 days was the time used in subsequent assays.

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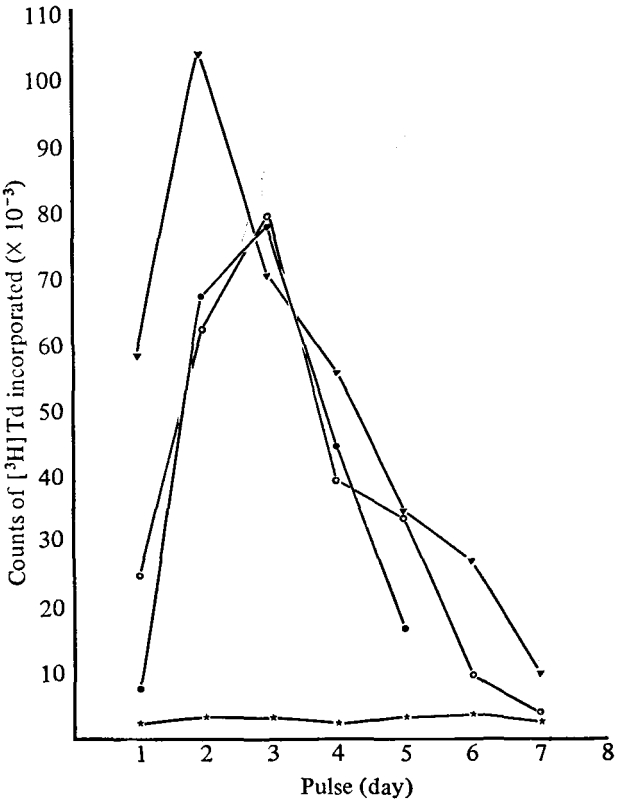


Fig. 3. Effect of pulse time on [$^3$H]thymidine incorporation. ●—●, ▼—▼, ⊗—⊗, Vaccinated animals; ◆—◆, control animal.

Table 1. *The effect of various reagents on [$^3$H]thymidine incorporation by PBL from vaccinated cattle*

| Antigen | [$^3$H]Thymidine incorporation* | | |
|---|---|---|---|
| | Vaccinated animals | | Control |
| Eagle's complete medium in contact with BHK for 24 h | 40498 ± 4377 | 20263 ± 2925 | 736 ± 154 |
| Eagle's complete medium | 3778 ± 578 | 1239 ± 158 | 478 ± 83 |
| Gradient treated BHK 24 h supernatant | 6327 ± 962 | 5230 ± 765 | 491 ± 67 |
| 24 h supernatant from RS cells | 3798 ± 729 | 1829 ± 207 | 245 ± 35 |
| BHK produced FMDV 140S antigen | 66822 ± 9277 | 26976 ± 4212 | 404 ± 98 |
| RS produced FMDV 140S antigen | 57861 ± 8210 | 29631 ± 3211 | 531 ± 102 |
| No antigen | 6988 ± 1097 | 951 ± 133 | 467 ± 28 |

* Mean of two separate experiments.

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### *Specificity of the [$^3H$]thymidine incorporation as a response to the 140S viral component*

Figures 2 and 3 show that PBL from unvaccinated animals have a very low level of [$^3$H]thymidine incorporation compared with those from vaccinated animals. Further controls using the reagents employed during the purification and inactivation of the 140S viral component all failed to give a response. In Table 1 however, we show that the supernatant from uninfected BHK cells caused some stimulation of PBL from vaccinated cattle but not those from uninoculated controls. Further studies, shown in Table 1, indicate that it was necessary for the Eagle's medium to have been in contact with the cells to give this effect and that, if the BHK supernatant was placed on a sucrose gradient and the bands taken that would have been equivalent to the 140S peaks, then the activity was lost. Supernatants from RS cells gave no response.

## DISCUSSION

The results of the study reported here indicate that bovine PBL from vaccinated animals do show a blastogenic response, as measured by [$^3$H]thymidine incorporation, to FMDV antigen. Lymphocytes from animals not previously exposed to antigen fail to be stimulated, supporting the concept that blastogenesis represents a specific immunological response by the PBL. Specificity studies further indicated that the blast cell response developed because of the presence of the 140S viral component.

We have also demonstrated by this technique that animals vaccinated with BHK-produced FMDV vaccine develop a response either to soluble BHK cell antigens or to a cell contaminant. Black (1977) has demonstrated the presence of serum reagins in cattle vaccinated with FMDV vaccines and our test provides evidence that a cell-mediated component may also play a part in the hypersensitivity reaction seen in some cattle vaccinated with FMDV.

PBL stimulation assays have been developed for a large number of viruses and they appear to provide an index of the cell-mediated immunity (CMI) response which an animal makes to a virus infection. The correlation, however, between humoral antibody levels and protection in FMD suggests that it is this limb of the immune system which is decisive in preventing reinfection. However, the demonstration of a blast cell response indicates that CMI mechanisms probably occur in response to inactivated antigen. To determine if this mechanism plays a significant part in protection it will be necessary to show that this response occurs quickly in naturally infected animals (perhaps before significant amounts of antibody have been produced) and that the blast cells can act as effector cells in preventing FMDV cytopathology.

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