# B LYMPHOCYTE ACTIVITIES IN THE OPOSSUM, *TRICHOSURUS VULPECULA*

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## ABSTRACT

The evolution of vertebrate immunity from the level of the protochordates to that of the metatherians is reviewed.

Using standard methods IgG, IgM and IgA were isolated from the serum or intestinal fluid of the Australian brush-tailed opossum, Trichosurus vulpecula. These were characterized in terms of their molecular weights, amino acid and carbohydrate compositions and values for their concentrations in serum were calculated. Two forms of IgG were seen which differed in their abilities to bind to insoluble matrices and also in their molecular weights. No antigenic differences were seen between them on analysis by agar diffusion . The molecular weight of the IgA seen in intestinal fluid and results from its analysis by agar diffusion suggest that the molecule may lack secretory component.

B lymphocytes were identified by their surface immunoglobulin and their complement and Fc receptors. The number of these cells in blood and various lymphoid tissues of T.vulpecula was found to be similar to the values reported for mice and humans. Lymphocyte fractionation on nylon wool columns confirmed that the markers employed were associated with an adherent cell population.

Blood lymphocytes were stimulated in vitro with a range of mitogens and the degree of transformation achieved with each was assessed by the cells' uptake of tritiated thymidine. Insoluble concanavalin A, pokeweed mitogen and lipopolysaccharide, in that order, were the most effective of the mitogens used on unfractionated blood lymphocytes. These three mitogens were further used in studies in which nylon wool fractionation of blood

lymphocytes was used to prepare B cell- and T cell-enriched cultures. Lipopolysaccharide was the only mitogen to stimulate B cells more than T cells. Insoluble concanavalin A consistently stimulated T cells to a greater extent than B cells as did pokeweed mitogen.

The ultrastructure of mitogen-stimulated cells was studied by electron microscopy and it was shown that lipopolysaccharide induced the formation of plasmablasts which resembled those of eutherians.

Mitogen-stimulated cells were also analysed for their production of immunoglobulins, the levels of de novo synthesised materials being measured by their incorporation of isotope-labelled leucine provided in the culture medium. Both secreted and intracellular proteins were measured in this way. Lipopolysaccharide, pokeweed mitogen and insoluble concanavalin A all induced significantly increased levels of 19S and 7S secreted proteins, these proteins being separated by gel filtration. Pokeweed mitogen induced the synthesis of significantly increased levels of both 19S and 7S intracellular proteins, while lipopolysaccharide and insoluble concanavalin A significantly increased the levels of 19S protein only. The presence of IgM and IgG in the 19S and 7S fractions was shown by their precipitation with class-specific antisera.

The immune responses of T.vulpecula to a particulate and a soluble antigen were compared with those of rabbits to the same antigens. Sheep erythrocytes, at two dose levels, were injected intravenously. The responses of opossums to $5 \times 10^9$ erythrocytes were appreciably more rapid than those of the rabbits. The responses of the two species to $25 \times 10^9$ erythrocytes were similar in the titres attained and the time taken to do so. The distribution of haemagglutinating activity between IgM and

IgG was studied and found to be essentially the same for both species for both levels of antigen. The responses of opossums to bovine serum albumin injected intramuscularly with Freund's adjuvants were similar to those of rabbits.

It is concluded that the B cell-dependent immune functions of T.vulpecula are as efficient as those of other metatherians and compare favourably with those of eutherians.

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## ABBREVIATIONS USED IN TEXT

| | |
|---|---|
| BGG | bovine gamma globulin |
| BSA | bovine serum albumin |
| Con.A | concanavalin A |
| cpm | counts per minute |
| CR | complement receptor |
| DEAE-cellulose | diethyl amino ethyl cellulose |
| DNP | dinitrophenyl sulfonilic acid |
| $DxSO_4$ | dextran sulphate |
| FcR | Fc receptor |
| FCS | foetal calf serum |
| HGG | human gamma globulin |
| IEP | immunoelectrophoresis |
| Insol.Con.A | insoluble concanavalin A |
| Insol.Con.A-LP | insoluble concanavalin A, laboratory prepared. |
| Insol.Con.A-W | insoluble concanavalin A, washed. |
| KLH | keyhole limpet haemocyanin |
| Lan.C | lanotoside C |
| LPS | lipopolysaccharide |
| 2-ME | 2-mercaptoethanol |
| MEM | minimal essential medium (Eagle's) |
| MLR | mixed lymphocyte reaction |
| MW | molecular weight |
| PBA | polyclonal B cell activator |
| PBS | phosphate buffered saline |
| PEG | polyethylene glycol |
| PHA | phytohaemagglutinin |
| PPD | purified protein derivative |
| Prot.A | protein A |
| PWM | pokeweed mitogen |
| SDS-PAGE | sodium dodecyl sulphate-polyacrylamide gel electrophoresis. |
| SI | stimulation index |
| SmIg | surface membrane immunoglobulin |

| | |
|---|---|
| SRBC | sheep red blood cells |
| TCA | trichloroacetic acid |
| TNP | trinitrophenyl sulfonilic acid |
| ZC | complement coated zymosan particles. |

## PREFACE

This study of some aspects of the immune capacity of Trichosurus vulpecula, the Australian brush-tailed opossum, can be justified on several grounds. Firstly, marsupials have been little studied from an immunological point of view, their geographical restriction making them unavailable to most investigators. Secondly, there is the relationship that Australian marsupials have to American marsupials, on the one hand, and to placental vertebrates on the other. Marsupials and placentals are believed to have evolved from a common therian stock, differentiation between the two lines beginning about 100 million years ago. The Australian marsupials are thought to be descendents of an American immigrant that dispersed across what is now Antarctica in the late Cretaceous period. Subsequently, Australian marsupials evolved in isolation for some 70 million years. It is therefore of interest to compare the immune capabilities of T.vulpecula, a relatively recent marsupial, with those of its ancient, unchanged didelphoid stock and with those of eutherian mammals. Thirdly, T.vulpecula has acquired a particular relevance to New Zealand. Since its introduction from Australia, it has flourished and conservative estimates put its present population in excess of 60 million. The species is involved in two zoonoses in this country, namely, tuberculosis and leptospirosis.

T.vulpecula seems highly susceptible to Mycobacterium tuberculosis, the disease being rapidly progressive in these animals. Foci occur throughout the country in which infected opossums, grazing bush-pasture fringes, maintain the infection in dairy cattle making eradication of tuberculosis from cattle difficult and presenting a health hazard to man. Furthermore, T.vulpecula shares its bush habitat with deer, animals of recognised economic potential. Tuberculosis is seen in captured wild deer and the

threat that this poses both to the health of the handlers of deer carcasses and to an expanding venison market does not need emphasising.

As regards to leptospirosis, up to 70 per cent of sexually mature opossums from farm land and 30 per cent from bush are infected with Leptospira balcanica. This organism has been reported as causing leptospirosis in man in Europe, but its relevance to the disease in New Zealand remains to be established.

Consequently, any information relating to the immune capabilities of the opossum could conceivably be useful in gaining a wider understanding of the epidemiology of these two diseases.

The present investigation examines some aspects of the immune competence of T.vulpecula. It is limited to B cell functions and considers the behaviour of these cells in vitro and in vivo and examines the immunoglobulins that they produce.