The Effect of Antigen Dose on the Carrier Effect: Studies on the Secondary *In Vivo* and *In Vitro* Anti-Hapten Plaque Forming Cell Response.

by

Robert C. McCarthy

Submitted to the faculty of the Graduate School
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
in the Department of Microbiology
Indiana University

November, 1976

Supervisory Committee

Joseph S. Ingraham	Microbiology (Chairman)
D.C. Bauer	Microbiology
Richard C. Bockrath	Microbiology
R.A. Jersild	Anatomy

Major:	Immunology
Minors:	Microbiology Life Sciences

This dissertation has been approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Microbiology, Indiana University Medical Center, Indianapolis, Indiana.

3 November 1976
Date

Joseph S. Ingraham, Ph.D.
Professor of Microbiology

D. C. Bauer, Ph.D.
Professor of Microbiology

Richard C. Bockrath, Ph.D.
Assistant Professor of Microbiology

R. A. Jersild, Ph.D.
Professor of Anatomy

ABSTRACT

Title: The Effect of Antigen Dose on the Carrier Effect: Studies on the Secondary *In Vivo* and *In Vitro* Anti-Hapten Plaque Forming Cell Response.

Previous *in vivo* studies with rabbits (*J. Exp. Med.* 126:1185 (1967) and *ibid* 127:717 (1968) showed that the carrier effect was partially or completely overriden when a hydrophobic hapten (dinitrophenol or trinitrophenol) coupled to hemocyanin or bovine serum proteins was used as the priming or challenge antigen. In both studies similar doses of antigen were used in the priming and challenge immunization (1 to 30 mg) and in some cases the best responses to heterologous conjugate were obtained if there was a prolonged interval between primary and secondary immunization.

The objective of the present studies was to examine the effect of antigen dose on the carrier effect in rabbits primed a year or more earlier with conjugated antigen. The conjugated antigens used in these studies contained the hydrophilic sulfanilazo hapten rather than hydrophobic haptens used in previous studies. The response to the homologous and heterologous conjugate was analyzed at the level of the antibody secreting cell by assaying for anti-hapten hemolytic plaque forming cells (PFC) in cells of regional lymph nodes of individual rabbits after *in vivo* or *in vitro* stimulation with hapten protein conjugates.

This system provided a more direct measure of the cellular events involved in the immune response and avoided the variability in priming that may occur between different animals.

Cells from the regional lymph nodes of individual rabbits primed with sulfanilazo-keyhole limpet hemocyanin (SA-KLH) or SA-bovine gamma globulin (SA-BGG) one to four years earlier were challenged either *in vivo* or *in vitro* with various doses of SA-KLH or SA-BGG. For *in vivo* studies, rabbits were challenged in one footpad with homologous conjugate and in the remaining footpads with various doses of heterologous conjugate. The doses of homologous conjugate were adjusted to give the maximal number of PFC and still restrict the response to the draining lymph node. Four and one-half days after challenge, the popliteal and midaxillary nodes were assayed for anti-SA PFC. Comparison of the indirect PFC responses in these nodes showed that heterologous conjugate was able to stimulate a PFC response that was 40 to 100% of the response found in nodes stimulated with homologous conjugate in 3 out of 6 animals. However, this response was obtained with a dose of antigen 300 to 30,000 fold higher than used in homologous challenge.

For *in vitro* studies, the popliteal or axillary nodes were removed from primed rabbits and cultured with different doses of SA-KLH or SA-BGG. Homologous conjugate gave maximal anti-SA indirect PFC responses at doses similar to those used *in vivo*. Heterologous conjugate gave at most 5% of the

response obtained with an optimal dose of homologous conjugate.

Thus, the results from these experiments show that the dose of heterologous conjugate used in challenge immunization was a major factor in overriding the carrier effect in rabbits. A model of the carrier effect is presented which proposes that it is due to the nature of T cell recognition.

Joseph S. Ingraham, Ph.D.

D.C. Bauer, Ph.D.

Richard C. Bockrath, Ph.D.

R.A. Jersild, Ph.D.

TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)