THE RESPONSE OF THE RETICULOENDOTHELIAL SYSTEM IN MICE TO TISSUE TRANSPLANTATION AND IMMUNOSUPPRESSIVE AGENTS.

by

Theo Gotjamanos, M.D.S. *(Adel.)*

A Thesis submitted for the Degree of Doctor of Philosophy in The University of Adelaide.

Department of Surgery
The University of Adelaide
December, 1970.

CONTENTS

SUMMARY

The phagocytic activity and morphology of Reticuloendothelial organs of Balb/c mice were studied following tissue transplantation and immunosuppressive treatment. Mice bearing medium-sized (4 cm^2) and massive (8 cm^2) skin isografts and allografts showed significantly increased blood clearance rates of opsonized *Salmonella typhimurium C5*. Increased clearance of colloidal carbon was also observed in mice bearing 1 cm^2 grafts, but mice with 4 cm^2 and 8 cm^2 grafts had normal phagocytic indices. The sequelae of severe surgical trauma may indirectly limit carbon clearance in mice with large grafts by depleting serum opsonins. Splenic enlargement occurred in isografted and allografted mice. The spleens of isografted mice showed increased erythropoiesis but little alteration in the lymphatic nodules and surrounding structures. Allografted mice showed increased granulopoiesis and erythropoiesis and cellular depletion from the marginal zones during the first phase of splenomegaly; a second peak of splenomegaly which occurred after allograft rejection was characterized by normal haematopoiesis but a marked enlargement of lymphatic nodules and germinal centres. Hepatomegaly paralleled the splenic enlargement in allografted mice and was due to enlarged hepatocytes which contained increased concentrations of RNA. The development of hepatomegaly following skin transplantation may represent a response which provides increased amounts of (a) purines, to sustain the intense cellular proliferation in lymphoid organs, and (b) plasma proteins, notably α - and β - globulins and fibrinogen which are elevated following surgery or trauma.

The use of large numbers of mice bearing different sized grafts permitted

a detailed study of the relationship between skin allograft size and survival time. In each of three H - 2 incompatible donor-recipient combinations tested, survival times of massive allografts were 2 to 3 days greater than those of small allografts, a difference that was highly statistically significant. The prolonged survival of massive allografts may be due to immunodepression following severe surgical trauma.

Intravenous administration of F_1 hybrid spleen cells to Balb/c mice resulted in hepatosplenomegaly and a marked increase in phagocytosis. In this *host-versus-graft* (HVG) situation, the order of increase in the phagocytic index for carbon was comparable with that reported for the *graft-versus-host* (GVH) reaction. Although the HVG reaction was characterized by changes in Reticuloendothelial organs also found in the GVH situation, *viz.*, phagocytic stimulation and hepatosplenomegaly, significant differences existed in the onset and nature of these changes. They occurred earlier in the HVG reaction and persisted for shorter periods; the relative number of liver macrophages did not increase and the spleen showed marked enlargement of lymphatic nodules with prominent germinal centre formation.

Cortisone in a dose of 10 mg/kg profoundly impaired carbon clearance by reducing the number of Kupffer cells capable of phagocytosing carbon. Hepatocytes in mice treated with 10 mg/kg of cortisone showed a 3-fold increase in lipid, while doses of 50 mg/kg and higher caused severe fatty change and hepatomegaly. When given daily over a 10 day period, 5 to 50 mg/kg of azathioprine did not affect phagocytosis, and phagocytic impairment occurred only when a near lethal dose (75 mg/kg) was used. Mild hepatotoxic changes followed

the administration of 25 mg/kg of azathioprine, while higher doses caused severe alterations in liver structure. Spleens from mice given azathioprine at 10 mg/kg and higher showed decreased red pulp haematopoiesis and histological abnormalities of lymphatic nodules.

One and two doses of rabbit anti-mouse antilymphocyte serum (ALS) depressed carbon clearance, while multiple doses stimulated phagocytosis and induced marked hepatosplenomegaly. The liver enlargement was due to hepatocyte hypertrophy, although increased numbers of Kupffer cells were also found in some cases. The enlarged spleens showed profound lymphoid cell depletion and intense haematopoiesis. The changes in phagocytic activity and morphology of Reticuloendothelial organs in adult Balb/c mice treated with ALS closely parallel those found in neonatally thymectomized mice. These results support the concept that ALS can confer a state of "immunological thymectomy" when administered to adult mice.

SIGNED STATEMENT

This thesis contains no material which has been accepted or submitted for the award of any other degree or diploma in any University. Furthermore, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of this thesis.

The work described herein has been the subject of the following publications:

GOTJAMANOS, T. (1970). The effect of skin allograft size on survival time following transplantation between mice differing at the H-2 locus.
Aust. J. Exper. Biol. Med. Sci. 48, 1-15.

GOTJAMANOS, T. (1970). A comparison of the changes produced in Reticuloendothelial organs of mice during host-versus-graft and graft-versus-host reactions.
Aust. J. Exper. Biol. Med. Sci., In Press.

GOTJAMANOS, T. (1970). Alterations in Reticuloendothelial organ structure and function following cortisone administration to mice.
RES, J. Reticuloendothelial Soc. 8, 421-433.

GOTJAMANOS, T. (1970). The effect of azathioprine on phagocytic activity and morphology of Reticuloendothelial organs in mice.
Pathology, In Press.

GOTJAMANOS, T. and GILL, P.G. (1970). Changes in phagocytic activity and morphology of Reticuloendothelial organs in mice induced by antilymphocyte serum.
Aust. J. Exper. Biol. Med. Sci. 48, 461-480.

Signed:

Theo Gotjamanos
27th November, 1970.

ACKNOWLEDGEMENTS

The work reported in this thesis was supported by grants from the National Health and Medical Research Council of Australia. I wish to express my sincere appreciation to the Head of the Department of Surgery, Professor J. Ludbrook for taking over the responsibility of supervising the latter part of my research programme, and for his valuable guidance in the preparation of the thesis.

Many other individuals assisted in various ways and I would like to thank the following: Professors R. P. Jepson and D. Rowley for making available laboratory facilities and for their interest in the investigations; Professor P. C. Reade for his initial supervision of the experiments on skin transplantation and bacterial clearance; Dr. T. Brown for writing the computer programme used to collate the data; Mr. W. Venables for performing the analysis of variance of graft survival times; Drs. I. Kotlarski and C. R. Jenkin for reading certain sections of the thesis and offering constructive criticism; Professor Sir Michael Woodruff for his interest in the early planning of the experiments; Professors J. C. Thonard and A. M. Horsnell for allowing me to use the photographic darkroom facilities in the Department of Dental Science and for their interest in the study; Dr. P. G. Gill for providing the antilymphocyte sera and antilymphocyte globulin preparations, and for his collaboration in the experiments with antisera; Dr. E. W. Witherspoon of Burroughs Wellcome and Co. (Australia) Ltd. for the generous supply of azathioprine; Mr. J. J. Darley for assisting in the preparation of tissues for electron microscopy; Mr. A. H. Chalmers for performing the biochemical estimations of liver RNA, DNA, protein

and glycogen; Mr. H. Schoemaker and Mrs. D. Cowling for assisting in the preparation of histological sections; Misses J. Watt, S. O'Brien, S. Weeks, G. Steinert and H. Jones for their care of the experimental animals; Mrs. J. Mitchell and Dr. D. Metcalf of The Walter and Eliza Hall Institute of Medical Research for their valuable information on splenic histology and the splenic changes induced by viruses; Drs. P.S. Russell and A.P. Monaco of the Harvard Medical School and Little, Brown and Company of Boston for allowing me to reproduce Table I : Terminology of Tissue Transplantation from "The Biology of Tissue Transplantation" (Russell, P.S. and Monaco, A.P., Little, Brown and Co., Boston, 1964); Mrs. I. Kronen for producing the line figures; Mr. J. Smith for photographing the grafted mice; Mrs. K. Smith for her secretarial assistance; Mrs. E. Walker for typing the thesis drafts and Mrs. D. Cowling for assisting in their proof reading; Mrs. C. Laing for typing the final thesis copy and Mr. G. Ashton for its duplication. To each of these persons, I am very grateful for their valuable and willing help.