ADVANCES IN
Immunology

VOLUME 25

EDITED BY

HENRY G. KUNKEL FRANK J. DIXON

ADVANCES IN
Immunology

EDITED BY

HENRY G. KUNKEL
The Rockefeller University
New York, New York

FRANK J. DIXON
Scripps Clinic and Research Foundation
La Jolla, California

VOLUME 25

1977

ACADEMIC PRESS *New York San Francisco London*

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 61–17057

ISBN 0–12–022425–9

PRINTED IN THE UNITED STATES OF AMERICA

LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

CLYDE F. BARKER, *Department of Surgery, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania* (1)

R. E. BILLINGHAM, *Department of Cell Biology, The University of Texas Health Science Center at Dallas, Texas* (1)

L. CHESS, *Division of Tumor Immunology, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts* (213)

DAVID L. GASSER, *Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania* (93)

MICHAEL POTTER, *Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland* (141)

S. F. SCHLOSSMAN, *Division of Tumor Immunology, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts* (213)

ANNE-MARIE SCHMITT-VERHULST, *Immunology Branch, National Cancer Institute, Bethesda, Maryland* (55)

GENE M. SHEARER, *Immunology Branch, National Cancer Institute, Bethesda, Maryland* (55)

PREFACE

The prediction that the seventies would be the decade of the lymphocyte clearly has been fulfilled. The science of this enigmatic cell no longer can be termed the new immunology, of interest only to a select few, but clearly has begun to permeate wide and diverse branches of biology. Detailed analysis of functional immunology at the cellular level has brought new insight into basic mechanisms of immunity. The application of this knowledge in specialized areas is apparent in much of the content of Volume 25.

The first article, by Barker and Billingham, on immunologically privileged sites, is especially useful because it brings together a diverse literature from a variety of specialized journals. Many intriguing questions are raised by the study of these privileged sites that are of obvious significance to the ordinary problems of transplantation. The uterus and the protection of the fetus during pregnancy continues to be one of the most challenging problems of immunology and this article is of considerable aid in placing it in proper perspective.

The paper by Shearer and Schmitt-Verhulst on histocompatibility restrictions in cell-mediated immunity is especially timely. One of the most surprising and significant developments stemming from the study of lymphocytes has been the elucidation of the relationship of most T-cell-mediated reactions to the histocompatibility system. This probably has been most thoroughly studied with respect to T-cell-mediated cytotoxicity and the authors have played a major role in this work. Similar conclusions have been reached in the three major systems analyzed: virally infected cells, chemically modified cells, and weak transplantation antigens. Controversy has developed as to whether one receptor or two are involved in the recognition of specific antigens and their associated histocompatibility types. It is an intriguing question which is well discussed in this review.

The paper by Gasser is a very complete review of immunogenetics in the rat. The primary aspect covered in special detail concerns the major histocompatibility antigens and their relation to immune response genes. It is in this area that the author himself has made important contributions. Immunoglobulin genetics is also a major topic and it is of special utility to have it accompany the histocompatibility section. Such additional topics as blood group immunogenetics are also well covered.

The article by Potter on the antigen-binding myeloma proteins of mice is an extremely thorough presentation of this important branch of

immunology. Just as the human myeloma proteins played such a significant part in the elucidation of antibody structure, the extensive work now going on with mouse myeloma proteins is providing key answers in V-region genetics. The hapten-binding proteins have been of special utility for these studies as well as for such others as X-ray crystallography for three-dimensional structure. Dr. Potter played an essential role in these developments, in considerable part due to his generous provision of these proteins to other workers.

The last article is by Chess and Schlossman on lymphocyte subpopulations in the human system. This topic is actually quite different from that developed in the mouse, largely because the usual markers obtained by interstrain immunization cannot be obtained similarly in the human. However, other systems which are well discussed in this review are available, as, for example, the T-cell characteristic of sheep cell rosette formation, which would be useful if similarly available in other species. Also included are a number of separation procedures for specific lymphocyte subpopulations, an area in which this laboratory has had wide experience.

H. G. KUNKEL
F. J. DIXON

CONTENTS

Immunologically Privileged Sites

CLYDE F. BARKER AND R. E. BILLINGHAM

Department of Surgery, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, and Department of Cell Biology, The University of Texas Health Science Center at Dallas, Texas

I. Introduction

Genetically alien grafts of a wide variety of both normal and malignant tissues have repeatedly been transplanted to anatomically unnatural sites in the body—i.e., transplanted heterotopically—for many different though frequently interrelated purposes. These include: (1) determination on an empirical basis of the site(s) most conducive to the growth and/or prolonged survival of a particular alien tissue in a normal host; (2) confirmation of endocrine function, or demonstration of graft responsiveness to hormones; (3) maintenance of a graft so that it can be visualized and its fate followed directly (for example, after transplantation to the anterior chamber of the eye) or, indirectly, by transillumination (after transplantation to the hamster's cheek pouch) without recourse to surgery; (4) ease of recovery; (5) studies on tissue

interreactions at the morphologic level; and (6) appraisal of the significance of some of the local anatomical and physiological variables for the healing-in of grafts and the elicitation and expression of transplantation immunity, by taking advantage of regional anatomical peculiarities, such as the absence of a lymphatic drainage system in the brain and of blood vessels in the anterior chamber, the cornea, and the lens of the eye.

The pertinent literature, spanning almost a century, is widely scattered and confusing because the experiments described were often inadequately controlled and conducted on ill-defined, heterogeneous stocks of animals by investigators who were usually unaware of the basic principles of transplantation immunology. However, the longevity undoubtedly enjoyed by alien grafts in some of the sites employed, as compared with that of similar grafts in other sites, taken in conjunction with the long-recognized and enigmatic success of a high proportion of both experimental and therapeutic orthotopic corneal allografts (Harris and Rathbun, 1972), have given rise to the concept that some of these sites may be "immunologically privileged" or favored—i.e., grafts transplanted to them are in some way partially or fully exempted from the normal rigors imposed by their histoincompatible status.

Contributing to the increased attention that has recently been focused on privileged sites are the following considerations: (1) Privileged sites can be created artificially. (2) It is recognized that better understanding of the modus operandi of privileged sites may lead to improvement in the results obtainable with therapeutic allografts. (3) There is a current search for a host site on which to test the pancreatic islet grafts that can now be prepared for treating diabetics without the need for immunosuppressive drugs (Barker, 1975). (4) Evidence exists that allografts sustained in some privileged sites may weaken on an immunologically specific basis the *host's* capacity to harm the alien cells concerned—producing a tolerant or "enhanced" status (see Billingham and Silvers, 1964). (5) Naturally occurring (as well as artificially created) privileged sites afford important "experiments of nature" pertinent to critical evaluation of the theory of immunologic surveillance against neoplastic disease (Burnet, 1970; Schwartz, 1975).

This article presents a critical account of the status of the known or suspected privileged sites in the body and evaluates their significance from both the immunologic and therapeutic viewpoints.

For completeness' sake, it may be stated, more or less empirically, that a few tissues can survive allotransplantation under conditions in which grafts of nearly all other tissues of similar genetic makeup

would suffer prompt rejection, i.e., there are also immunologically privileged tissues, most notably trophoblast and its malignant derivative, choriocarcinoma, and cartilage (see Beer and Billingham, 1976; Heyner, 1973).

Nude mice deserve a mention here because their basic congenital athymic status renders them "immunologically privileged" hosts that sustain on an indefinite basis both allografts and xenografts from a wide spectrum of vertebrate donors (Manning *et al.*, 1973; Rygaard, 1973).

II. The Anterior Chamber of the Eye

Use of the anterior chamber as a graft site was pioneered by Van Dooremaal (1873) and by Zahn (1884), who observed short-term survival of human malignant tumor tissue and a higher degree of survival with fetal cartilage from both allogeneic and xenogeneic donors in the anterior chambers of rabbits' eyes. Subsequently, Hegner (1913) reported the short-term growth before regression of mouse tumor tissue grafts in the anterior chambers of rats, mice, guinea pigs, and rabbits, although he had little success with human tumor material in rats' eyes. By contrast, Smirnova (1937) and Greene and various co-workers (see, e.g., Greene, 1952, 1957; Greene and Arnold, 1945; Greene and Murphy, 1945), on the basis of very extensive studies, reported the growth and long-term survival of a variety of human tumors that had acquired the capacity to invade and metastasize in the anterior chamber of rats, guinea pigs, and rabbits. In Greene's experience, once xenogeneic tumors had become established in the anterior chamber, it was often possible to maintain them by serial transplantation within the eyes of other members of the initial host species, and sometimes they could be successfully transferred to the testis. However, neither benign nor malignant tumors at an early stage of their development survived heterotransplantation to the eye. Human melanomas, the slowest growing of the tumors studied, when transplanted to rabbits' eyes, sometimes persisted apparently unaltered for several months before growth occurred. On the basis of these and other findings, Greene maintained that heterotransplantability could furnish the basis of a biologic test of malignancy. In his hands, unlike normal adult tissue, embryonic tissue and neoplastic brain tissue, which does not metastasize, also survived both xenogeneic and allogeneic transplantation.

However, it is important to note that Greene's interesting findings on the xenotransplantation and allotransplantation of malignant and embryonic tissues have not been reproducible in the hands of many,

indeed the majority of, other investigators (Morriss *et al.*, 1950). In extensive studies on allografts of various normal tissues from fetal and adult donors transplanted to the anterior chambers of mice, Browning (1949) found that, after an initial phase of growth, regression in the fourth week was the invariable fate of the grafts. A possible complication of his experimental design was the use of *both* eyes in each host. Furthermore, no experiments were conducted to determine the fate of similar grafts in other sites in the body. Dameron (1950, 1951) was much more successful with a variety of fetal endocrine tissue allografts in the eyes of guinea pigs and rats, especially in hosts previously rendered totally deficient in the endocrine tissue concerned. Histologic evidence of maturation of the endocrine tissue after transplantation was accompanied by functional evidence of its survival. Indeed, one could cite many investigators who have used the anterior chamber with a reasonable degree of success to sustain, for a variety of experimental purposes, endocrine, gonadal, and other tissues from immature and adult allogeneic donors. Markee's (1932) observation that endometrial tissue allografts in the anterior chambers of guinea pigs, rabbits, and monkeys rapidly acquired a blood supply and underwent estrous cycles for long periods is a familiar classic of reproductive endocrinology. Working with outbred guinea pigs, Woodruff and Woodruff (1950) found that 78% of thyroid tissue allografts in the anterior chambers of thyroidectomized hosts quickly became vascularized, increased in size and survived permanently, evoking little or no inflammatory reaction. By contrast, only 11% of thyroid allografts transplanted subcutaneously in similar hosts were successful. Of particular interest were the authors' observations that (1) intraocular allografts gradually lost their initial susceptibility to specific sensitivity elicited in hosts by a subsequent subcutaneous thyroid tissue allograft from the original donor; and (2) when long-established intraocular grafts were recovered and transplanted to a subcutaneous site in the *same* host, they became vascularized and survived indefinitely in a high proportion of instances. These findings indicated that some kind of adaptation must have taken place, either in the grafts themselves or in their hosts—the Woodruffs favored the latter possibility.

The present authors have been unable to show that, in hamsters and guinea pigs, skin allografts sustained by the cheek pouch milieu or by the alymphatic skin pedicle flap, respectively, either: (a) weaken the host's capacity to respond to subsequent orthotopic skin allografts from the original donor strain, or (b) undergo some kind of antigenic attenuation, possibly as a consequence of the loss of passenger leuko-

cytes (see Billingham, 1971; Talmage *et al.*, 1975). However, Warden *et al.* (1973) have confirmed and extended the observations of the Woodruffs in a study that entailed transplantation of DA strain rat thyroid tissue allografts to Ag-B locus incompatible, thyroidectomized FI strain hosts. They recovered long-established intraocular grafts and compared their survival after subcutaneous implantation into the original hosts and into normal rats syngeneic with the original hosts. A functional criterion of allograft survival—serum thyroxine levels in the thyroidectomized hosts—was used. Only the grafts in the first group survived, indicating that adaptation must have taken place at the level of the *host*, rather than the graft. These authors suggested that active immunologic enhancement (see Brent and Kilshaw, 1976) of the host was responsible for weakening its reactivity to the subcutaneous allograft. Consistent with these findings is a report that thyroidectomized and parathyroidectomized hamsters bearing thyroid and parathyroid allografts, respectively, of 50–60 days' standing in their anterior chambers displayed weakened reactivity when tested with orthotopic skin grafts from the same alien donor strain (Weiner, 1965). Evidence will be presented below (see pages 7 and 8) that lends strong support to the concept that the long exemption from rejection that may be enjoyed by intraocular allografts depends upon some kind of induced suppression of the host's capacity to mount a cellular immune response.

Medawar and Russell (1958) demonstrated that a significant proportion of adrenalectomized mice can subsist for at least several weeks upon allografts of adrenal cortical tissue in the anterior chamber. The fate of skin allografts in the anterior chamber has been studied by several investigators, but, as with other types of grafts in this site, the results are enigmatic because of inconsistency. All investigators are in agreement that skin grafts, like most other tissue grafts in this site, become revascularized within a day or two, usually from the iris. In 1948, in a study of the role of blood and lymph vessels in transplantation immunity, Medawar (1948) reported that skin allografts transplanted to the anterior chambers of specifically *immunized* rabbits were destroyed if, and only if, they were revascularized. Browning (1949) observed that skin allografts in the anterior chambers of mice were rejected within 30 days, whereas in guinea pigs, according to Connelly (1961), skin allografts grew successfully in a high proportion of subjects, there being no difference in histologic appearance between autografts and allografts of 65 days' standing. Despite the fact that animals bearing anterior chamber grafts rejected orthotopic skin allografts from the original donor in an accelerated manner, the in-

traocular grafts responsible for the sensitization continued to survive. In the anterior chambers of rabbits' eyes, skin allografts consistently survived for long periods of time in Raju and Grogan's (1969) experience, whereas Franklin and Prendergast (1970) found that rejection was always complete by postoperative day 10 as a consequence of a typical allograft reaction. Telling observations substantiating the immunologic basis of this rejection were the indefinite survival of intraocular skin autografts and the prolongation of survival of intraocular skin allografts in rabbits previously exposed to 500 r whole-body irradiation.

Recently, Vessella *et al.* (1974) and Kaplan and various associates Kaplan and Stevens, 1975; Kaplan *et al.*, 1975a,b) presented the findings of critical systematic analyses of the transplantation immunology of the anterior chamber of the eye, using inbred strains of rats. Their findings go some way toward explaining the highly variable results obtained by other investigators. Although the expectation of survival of intraocular skin allografts significantly exceeded that of orthotopic controls, the immunogenetic disparity between donor and host was an important variable—Ag-B locus compatible grafts living longer than Ag-B locus incompatible grafts. Graft size, or dosage, was another important variable, smaller grafts surviving longer than larger ones. Thyroid tissue allografts enjoyed less protection than skin in the anterior chamber, especially in euthyroid hosts, and various findings sustained the authors' conclusion that the high degree of susceptibility of thyroid tissue to ischemic necrosis appeared to magnify its immunogenicity. The capacity of a thyroid tissue allograft in one eye to curtail the survival of a concomitantly transplanted skin allograft from the same donor in the opposite eye was indicative of the ability of anterior chamber thyroid grafts to elicit systemic immunity.

Additional evidence that, despite the privilege which the anterior chamber can extend to allografts, this site is certainly not lacking in an immunologically significant afferent connection with the animal's immune response machinery is afforded by the following observations: (1) Three weeks' residence, in a viable condition, of Lewis (LE) strain skin in the eyes of Fischer (FI) strain rat hosts sensitizes them in respect of orthotopic Lewis (LE) strain test skin grafts. (2) FI hosts of intraocular DA strain skin develop significant hemagglutinin titers as early as 21 days after transplantation (Kaplan and Stevens, 1975).

Kaplan *et al.* (1975a) have shown that, when parental strain lymph node cells are inoculated into the anterior chambers of genetically tolerant F_1 hybrid rats, graft-versus-host (GVH) reactions develop that are expressed as an anterior uveitis. These reactions resemble GVH

reactions incitable locally in other sites, but with one important exception. Animals that recover from systemic GVH disease usually become refractory to subsequent rechallenge with lymphoid cells from the original donor. This also applies to the local GVH reactivity that underlies the popliteal lymph node assay in the rat (see Grebe and Streilein, 1976). However, (FI × DA)F_1 hybrid rat hosts that had developed primary intraocular GVH reactions as a consequence of inoculation with 10×10^6 parental strain lymph node cells *were* able to develop GVH reactions in their local popliteal lymph nodes following subsequent rechallenge with parental strain node cells in their hind foot-pads. Likewise, animals that had given a primary GVH reaction in one eye responded by fulminant GVH reactions when rechallenged in either the same or the other eye. The authors tentatively ascribed this disparity in refractoriness, following popliteal and anterior chamber GVH reactions, respectively, to the absence of a lymphatic drainage in the anterior chamber which forces antigen, or antigen-reactive cells introduced into it, to leave exclusively via the intravenous route.

Local GVH reactions were also used by Kaplan and Streilein (1974) further to define the pathway by which antigenic material or lymphocytes can escape from the anterior chamber. Viable suspensions of lymph node cells from FI rats sensitized to DA rat tissue antigens were injected into either the subconjunctival space or the anterior chamber of genetically tolerant (FI × DA)F_1 hybrid hosts. Hypertrophy of the cervical nodes and splenomegaly were incited by the former, but not the latter, inocula, substantiating evidence from dye injection studies that the anterior chamber has no lymphatic drainage. Nevertheless, slit-lamp microscopy strongly indicated that intraocular lymphocytic cellular inocula disappear within a few days.

Evidence that these cells enter the host's blood circulation and can profoundly influence its central machinery of immunologic response was provided by observations that (1) anti-DA strain hemagglutinins appeared within 4 days of inoculating the anterior chambers of normal FI strain rats with (FI × DA)F_1 hybrid lymphoid cells; (2) (FI × DA)F_1 hybrid test skin grafts enjoyed a few days' prolongation of survival on FI rats that had received an intraocular or an intravenous inoculation of hybrid lymphoid cells 10 days beforehand, whereas similar skin grafts placed on subconjunctivally inoculated hosts underwent summary rejection; and (3) DA rats that had been injected intraocularly with FI strain node cells developed high titers of anti-FI lymphocytotoxic antibodies in addition to hemagglutinins. Furthermore, test skin allografts on these animals were rejected in an *immune*

manner, in contrast to the prolongation of skin graft survival seen in the animals which had received an intraocular injection of F_1 hybrid node cells. This, according to Kaplan and Streilein (1974), indicated that the recipient's immunologic response to the alien lymphocytic inoculum in its anterior chamber depended upon the immunologic reactivity of these cells vis-à-vis the host.

Two well-established key facts about the spleen—first, that, by virtue of its size and blood flow, it receives and processes most of the antigenic material administered to an animal by the intravenous route; and, second, that it is the principal source of "enhancing" antibodies—were taken into consideration by Kaplan and Streilein (1974; see also Streilein *et al.*, 1975a) when they postulated that the essential quality of the anterior chamber, and possibly some other immunologically privileged sites apparently devoid of lymphatic drainage, is their ability to allow antigen *direct* access to the blood stream, bypassing peripheral nodes altogether. The resultant intensive exposure of hosts to antigen via their spleens may then favor the development of unresponsiveness (tolerance and/or enhancement) rather than sensitivity, as a consequence of the synthesis of tissue-protecting enhancing antibodies, the generation of suppressor T lymphocytes (Asherson and Zembala, 1976), or the selective trapping of antigen-reactive cytotoxic lymphocytes within the spleen (Streilein and Read, 1976). Their finding that inoculation of splenectomized FI rats, via the anterior chamber or intravenously, with (FI × DA)F_1 hybrid lymphocytes not only failed to prolong the survival of subsequent test skin allografts from the hybrid donors—indeed, it tended to curtail their survival as compared with controls—sustains this interesting concept.

The route by which cells introduced into the anterior chamber gain access to the host's blood stream has yet to be defined. The obvious possibilities are via the blood vessels supplying the ciliary body and/or the canal of Schlemm. Whether open-ended, epithelial-lined canaliculi run from the anterior chamber into this canal is still equivocal (see Kaplan *et al.*, 1975b).

III. The Cornea

The relatively high degree of success that has long been known to attend the use of penetrating corneal allografts to achieve the repair of corneal lesions in the eyes of nonimmunosuppressed patients and the even greater success rate of similar grafts in the *normal* eyes of experimental animals have long been recognized as apparent exceptions to

the "laws of transplantation," posing the questions whether corneal tissue is effectively nonantigenic, and whether the cornea as a graft site has unique properties.

That corneal tissue is effectively antigenic has repeatedly been demonstrated. For example, when transplanted heterotopically to vascularized beds, such as subcutaneous pockets or full-thickness skin defects in rabbits, corneal allografts become vascularized and both elicit and succumb to transplantation immunity just as do skin grafts (see Billingham and Boswell, 1953). Furthermore, allografts of corneal epithelium growing on extensive vascular beds prepared by removal of the full thickness of the skin are also rejected like grafts of pure epidermis (Billingham and Boswell, 1953; Khodadoust and Silverstein, 1966). Khodadoust and Silverstein (1969) have developed an ingenious method for transplanting allogeneic corneal epithelium, stroma, or Descemet's membrane plus endothelium to the host's cornea. When vascularization of the recipient bed was procured by positioning the graft eccentrically near the limbus, or by delayed removal of the sutures, each type of graft sensitized the host and underwent rejection. Finally, it has been shown that successful, recently transplanted penetrating corneal allografts in rabbits are vulnerable to transplantation immunity generated by transplantation of donor skin grafts to the host, but this susceptibility on the part of corneal allografts is usually lost with time (Maumenee, 1951).

The special privilege that orthotopic corneal allografts appear to enjoy cannot be ascribed to surreptitious replacement of alien donor cells by equivalent cells of host origin. Experiments of appropriate design, making use of the sex chromosome marker, karyotype analysis, tritiated thymidine and other labeling techniques, have established unequivocally that in a successful penetrating cornea allograft, there *is* long-term survival of epithelial cells, keratocytes, and endothelial cells (see Harris and Rathbun, 1972). In corneal grafts that have been stored, the epithelium frequently does become totally detached, in which case it is promptly replaced by centripetal migration of epithelium of host origin, but this does not prejudice the success of the graft. However, the presence and continued viability of the original endothelium appear to be mandatory both for the initial and for the continued success of penetrating corneal allografts because of the great physiologic dependence of the entire cornea on the integrity of this layer. One of its functions is to act as a barrier to the imbibition of fluid from the aqueous humor, as well as a metabolic pump that dehydrates the stroma. Lamellar, i.e., partial thickness, allografts appear