FROM THE DEPARTMENTS OF RADIOLOGY AND SURGERY, MEDICAL COLLEGE OF VIRGINIA, RICHMOND, VIRGINIA, U.S.A.

RADIATION FOR IMMUNOSUPPRESSION IN HUMAN ORGAN TRANSPLANTATION

I. Experimental data

by

S. H. LEVITT, F. T. O'FOGHLUDHA, R. L. ROYSTER, J. S. WOLF, R. R. LOWER and L. S. DEGIORGI

The replacement of diseased or worn out parts or organs has intrigued man for centuries. Mention is made in Greek mythology of transplants from animal to man, and early Christian legends and folk tales tell of successful transplants of noses and limbs from one individual to another. In the middle ages physicians and lay literary figures repeatedly considered in their treatises the possibility of transplantation of a structure from one individual to another.

Probably the first successful clinical transplant in modern history was reported in 1823 by BUNGER (the historical information in this section was treated in detail by CONVERSE & CASSON in 1968) who reconstructed part of a woman's nose by a free graft from her thighs. As early as the middle of the 19th century the difference between the behavior of autografts, allografts and xenografts was recognized by BECK, one of the early workers in transplant research.

The types of tissue transplants are given below:

Autochthonous (autologous)	Individual's own tissues
Syngeneic (isologous)	Identical twins; animal inbred strain
Allogeneic (homologous)	Same species
Xenogeneic (heterologous)	Different species

Submitted for publication 11 May 1970.

The idea that allograft rejection is mediated by a process of active immunity is attributed to JENSEN (1903). Further work to verify this concept was done by many other investigators. The first organ transplant was reportedly done by BECK in Chicago in 1903, at which time he transplanted an experimental kidney. Further developmental transplantation approaches were carried out by CARREL, FLORESCO, DECLEVERS and others.

The basic concepts concerning the reaction of a host to transplantation of tissue from another unrelated individual were first formulated by investigators in the field of tumor research early in the 20th century. The hypothesis of the immune response was one of those proposed to explain the rejection phenomenon and was expounded by RUSSEL and MURPHY. MURPHY concluded from a series of experiments utilizing transplanted tumors that the defense mechanism responsible for the destruction of the transplant was centered in the small lymphoid cell. He later attempted to modify the rejection process by interference with the function of the lymphocyte through irradiation and the administration of benzoyl. Despite this work, there remained much disagreement about the role of immune response in the allogenic tissue transplant reaction until the mid 1940's. In 1944 MEDEWAR carried out the basic experiments which led to the recognition of the role of the immune response in transplant rejection and incidentally to further exploration of the role of radiation in the suppression of this response.

The basic problems in organ transplantation have been and are the technical factors involved in organ transplant preservation and the prevention of the rejection phenomenon. This paper will deal with the role of radiotherapy which is primarily that of preventing rejection in organ transplantation.

Types of rejection

Rejection of organ transplants is classified under three headings: hyperacute, acute, and chronic.

Hyperacute reactions occur within minutes or a few hours after the organ has been transplanted and are very similar in timing to second-set transplant rejections. Changes in the organ may frequently be observed before completion of the transplant operation. These include blood vessel destruction, hemorrhage and thrombosis (WILLIAMS et coll. 1968). This immediate reaction is mediated by humoral antibodies present in the recipient prior to the transplant surgery. These antibodies may develop as a result of pregnancies, blood transfusions, previous transplants, or in certain instances, bacterial infection.

Acute rejection occurs within a few weeks and up to about four months following transplant. This reaction is the classical primary response of cellular immunity, which proceeds in some patients regardless of the method of immuno-

suppression used. An infiltration by monocytes is the first demonstrable pathologic phenomenon and occurs within 24 to 48 hours. Endothelial cellular damage, interstitial edema, and round-cell infiltration, tubular necrosis and occlusion of vessels and decreased renal blood flow, are regular features of acute rejection.

It is believed by most authorities that the small lymphocyte is responsible for acute rejection. The role of the lymphocytes in the process is hypothesized as follows: small circulating lymphocytes, called immunologically competent small lymphocytes (ICSL), react with the challenging antigen and migrate via afferent lymphatics to the lymph nodes (this phase is sometimes referred to as the afferent arc). In the lymph nodes these lymphocytes become fixed in lymphatic tissue and differentiate into large pyroninophilic cells which go through a number of divisions and result in the production of plasma cells or new small lymphocytes. These new cells are also called the immunologically activated small lymphocytes (IASL). These activated cells migrate in time to the graft and in some manner, not yet completely understood, destroy the graft tissue (this phase is called the efferent arc).

The possible methods of action (CRONKITE & CHANANA 1968) of these sensitized cells in the graft are: (1) having antibody-like structures bound into them, acting directly on target cells, (2) releasing a humoral antibody in high concentration near the cells, (3) carrying a cytophilic antibody, (4) acting as phagocytic scavengers after the graft has been opsonized by humoral antibody circulating in the plasma, and (5) releasing enzymes which damage both graft and host cells.

Chronic rejection occurs after about four months, and in certain instances as long as four years. It is seen in patients on immunosuppressive therapy and may demonstrate a partially successful attempt at immunosuppression. Cellular infiltration is absent, and histologic changes occur in the basement membrane of the glomerulus and the endothelium of small blood vessels. Endothelial proliferations is observed with narrowing and ultimate occlusion of the lumina of vessels. Damage to glomerular capillaries leads to protein loss in the urine, a slow rise in blood urea nitrogen (BUN) and serum creatine and a decline in glomerular filtration and renal plasma flow. This rejection phenomenon is apparently produced by circulating humoral antibodies (immunoglobulins). These antibodies have been detected in man following removal of kidney allografts (HUME & WOLF 1967).

At present, only the acute form of rejection can be practically controlled although a great amount of research is going into attempts to control the hyperacute and chronic types. The role of irradiation (and indeed all other forms of immunosuppressive therapy used at this time) is to suppress the acute form of rejection.

Present methods of immunosuppression

Immunosuppressive agents such as steroids, radiation, and antimetabolites, together with antilymphocyte serum and globulin 'have been used, alone or in various combinations, to suppress the immune response in man'.

All the measures commonly used today depend upon the destruction of the lymphocyte and suppression of new lymphocyte production or at least deactivation of its role in the immune response. Since humoral immunity against the transplanted organ also develops and free antibodies may be present, the antilymphocyte actions of prednisone, azathioprine, antilymphocyte globulin or radiation may not be completely effective.

The chemotherapeutic agents used most commonly are prednisone and azathioprine. The routine daily doses given post-transplant are 3 to 4 mg/kg of azathioprine and 5 to 60 mg of prednisone depending upon graft acceptance.

Radiation has been used in various ways and with various doses. Radiation acts to destroy the lymphocytes that are present and to suppress new production, thus interfering with their role in the launching of the rejection process in allotransplanted tissue. The small lymphocyte is very sensitive to radiation; the D_0 for all proliferating hematopoietic cells is about 100 rad, and it has been shown that doses as small as 12 to 50 rad can destroy a fraction of the lymphocytes.

Methods of irradiation

Total body irradiation

The purpose of total body irradiation is to suppress all immune mechanisms. Total body irradiation in doses sufficient to suppress humoral immune response substantially or to increase the acceptance of allografts, results in an almost complete loss of the small lymphocytes and destruction of germinal centers in the lymph nodes and spleen. The population of small lymphocytes in the bone marrow diminishes along with the other cell lines.

A. Supralethal irradiation with bone marrow transplantation. Irradiation of the host prior to transplant in order to prevent rejection requires doses in man which have been imcompatible with survival for the most part.

B. Sublethal total body irradiation. This has not been successful in most animal experiments unless immunosuppressive agents are also used. In man, it has been used in the first four kidney transplants at our institution; two of the patients later died, and two are still alive. Because of the dangers inherent in this approach, it has been abandoned.

Local irradiation

The purpose of local irradiation is to depress immune response and lymphocytes locally and to avoid the reaction inherent in total body irradiation.

A. Irradiation of the site prior to transplant. In skin grafts, the antigen-sensitive lymphocytes draw from graft site to the local nodes for ICSL development so that irradiation of these areas leads to prolonged skin graft survival. However, local kidney transplants do not necessarily depend upon regional lymphatics or lymph nodes for ICSL development and irradiation of the graft site prior to kidney transplant is not effective. This difference in route of sensitization was shown in the surgical laboratories of the Medical College of Virginia where complete isolation of a kidney transplant from regional, lymphatic drainage did not prolong survival (HUME et coll. 1963). WOODRUFF et coll. (1963) also found that irradiation of the graft site prior to transplant in human renal transplants was not effective in preventing rejection.

B. External irradiation of the transplant. BANKS et coll. (1961) found that local irradiation in kidney transplants of dogs did not prolong functional survival when doses of up to 770 rad were given in daily fractions of 100 rad. KAUFFMAN et coll. (1965) found that 150 rad was the smallest individual dose capable of preventing homograft rejection; this was most effective when given on the day of transplant. A dose of 200 rad given on the initial day appeared to be less effective in prolonging graft survival. The possible mechanisms are:

Alteration of graft antigenicity is not likely since single doses as high as 1500 rad to dog kidneys prior to transplant did not decrease the antigenicity.

Alteration of graft bed and adjacent lymphatics may be a factor in prevention of skin graft rejection but not in kidney grafts because of the difference in afferent and efferent pathways in the two situations.

Alteration of graft versus host activity. It has been theorized that local irradiation might act to decrease graft versus host reaction. However, FOWLER & PORTER discount this idea since they have definitely shown that the cells infiltrating the kidney are of host origin (CRONKITE & CHANANA 1968).

Local non-specific anti-inflammatory effect.

Interference with the afferent and efferent arcs. Radiation destroys the sensitized host lymphocytes invading the transplant, thereby interfering with the efferent arc rejection and thus leading to prolonged graft survival. This has been confirmed by experiments in which two kidneys from a single donor were placed into a single recipient. One kidney was irradiated and the other was not, and the suvival was significantly greater in the irradiated kidney.

Local roentgen irradiation to the transplant is also capable of affecting the afferent arc of the immune response. In another series of experiments involving second transplants from the same donor into the same recipient, a series of dogs were immunized by a kidney transplant followed a week later by a second transplant. The second transplant was rejected in an accelerated manner. In another series of dogs the first transplant was irradiated. Following rejection of this kidney

the second transplant was done. This second transplant was also rejected but not as rapidly. The difference in survival was significant. This showed that irradiation had partially interfered with immunization of the host.

That local irradiation is also useful in reducing heart rejection in dogs has been shown by LOWER and associates (GRAHAM et coll. 1970, LOWER et coll. 1968) who studied fifteen animals that had undergone successful cardiac allotransplantation. All dogs received a course of azathioprine for the first three days post-transplant and were treated with ^{60}Co gamma rays through 10 $\times$ 12 cm portals while lying on their right sides. Various dose schedules were used (see below), all doses being calculated at 6 cm depth in the para-axillary area. The electrocardiogram was monitored daily in all animals. Acute rejection was diagnosed if the QRS voltage fell to one-half or less of the maximum post-operative level while the response was considered favorable if the voltage returned to normal or nearly normal values. At the completion of the irradiation, open biopsy of the left ventricle was carried out in all animals and light and electron microscopic investigations were performed.

Two groups of animals were studied. Group 1 consisted of three dogs receiving 450 rad in three equal consecutive daily fractions, one dog which received 600 rad in four such fractions, and one which received 900 rad in six fractions. Only one animal in this group showed any electrocardiographic response to irradiation and none showed any improvement microscopically.

In Group 2 the total dose was considerably larger (1 500 rad in 300-rad fractions given on five consecutive days) than in Group 1. Six of ten animals treated at this larger dose level responded to radiation as demonstrated both in the ECG and histologically. In five of the six animals which improved there was marked decrease in the degree of cellular infiltration, interstitial edema, and congestion, and there was less endothelial-cell swelling and necrosis. Ultrastructurally, the outstanding change was in the myocytes. Prior to radiation these cells had a smudgy and disoriented appearance, the transverse bands were frequently indistinct and the mitochondria were swollen and vesicular, and myelin figures and lipid droplets were readily apparent. There was hypertrophy of the endothelium of the vessels, with interstitial edema and monocyte infiltration. Following irradiation, the myocytes appeared more normal, were less disoriented, and the transverse bands were more distinct. The mitochondria, endoplasmic reticulum and transverse tubular system were also less vesicular, swelling of the endothelium in the small blood vessels was less marked and there was less cellular infiltration of the interstitium.

The experiment is most noteworthy since this is the first time that the therapeutic effect of radiation on acute rejection has been documented histologically. The study also seems to demonstrate that in order to prevent rejection of heart

transplants higher dose levels of irradiation are needed than in the case of kidney grafts.

C. Internal radiation of the graft using isotopes. WILLIAMS & SCHAPIRO gave dogs radioactive chlormedrin ^{203}Hg intravenously and noted prolonged survival of renal homografts when doses of approximately 85 to 160 rad per day were delivered by the material. The difficulties of controlling localization and dose are obvious, and this approach does not appear to be useful in man.

D. Blood irradiation. Irradiation of the circulating blood has been utilized to produce generalized lymphopenia and thereby diminish homograft rejection by decreasing ICSL without the reactions of total body irradiation.

Irradiation of an artery leading to the renal homograft. At the surgical laboratories of the Medical College of Virginia a skin pedicle was created in the neck of a dog and the carotid artery was incorporated in it. Next, a renal transplant was carried out and the distal end of the carotid skin pedicle was anastomosed to the new artery of the transplanted kidney. The carotid artery was then irradiated by a shielded radiation source. There was subsequent prolongation of renal homograft survival. Since a generalized lymphopenia was also produced, it may be that although only the blood to the transplant had been irradiated, enough of the blood volume had been irradiated to produce a general extracorporeal irradiation rather than selective irradiation of blood entering the kidney.

Intra-arterial implants. Implantation of sialastic coated pellets of ^{90}Y into the center of the abdominal aorta of dogs prolonged renal homograft survival in our laboratories. Serial histologic sections of the spleen and mesenteric lymph nodes showed progressive depletion during the radiation period.

Extracorporeal blood irradiation. CRONKITE & CHANANA demonstrated prolongation of skin homografts using an extra-corporeal shunt exposed to ^{60}Co gamma rays. Although this method is quite effective, the heavy shielding required makes the equipment very cumbersome.

Shunt irradiators incorporating pure beta-emitters such as ^{90}Sr or ^{32}P have the advantage that the necessary shielding is very much lighter because the beta particles have low penetrating power. This otherwise advantageous property of the beta rays is, however, a drawback if irradiation of large blood volumes is attempted because only those portions of the blood passing very close to the radiation source receive any energy from it. When the volume of blood instantaneously irradiated is small, as dictated by this last limitation, prolonged irradiation becomes necessary in order to achieve any substantial uniformity of dose, because a given cell passes through the shunt at irregular intervals and a wide distribution of cell doses therefore occurs for the shorter exposure time (O'FOGHLUDHA 1969).

Suitable beta-irradiators have been constructed in several laboratories and at

least one model is commercially available; the Departments of Radiology and Surgery at our institution have collaborated in producing and testing several successful devices, one of which is a wrist-borne bracelet containing a coil of thin-walled plastic tubing attached to an a.-v. shunt near the wrist (O'FOGHLUDHA & WOLF 1968). Curved plates incorporating sealed ^{90}Sr sources bear against the coil and transfer approximately one-quarter of the radiated energy to the blood traversing the tubing. The device is completely portable and may be worn by the patient for periods long enough to insure that the blood dose is reasonably uniform. There is no appreciable external hazard (O'FOGHLUDHA) provided the containers remain intact. The device can be worn by an ambulant patient who must, however, stay in the hospital. The current device can deliver a mean dose of approximately 1 000 rad/day to patients of normal blood volume. A pilot irradiator relying on ^{32}P has also been constructed and further work continues (O'FOGHLUDHA).

Experimentally, the beta-ray method has been shown to prolong renal homograft functional survival in humans most effectively when irradiation is begun immediately after transplantation (O'FOGHLUDHA).

E. Lymphatic or reticulo-endothelial cell irradiation. CRONKITE & CHANANA studied the effect of thoracic duct lymph irradiation on skin graft placed so that they drained almost entirely into the thoracic duct. Skin grafts placed in other areas so that the lymphatic drainage was not solely to the thoracic duct, did not show the same increased survival. The authors felt that this phenomenon demonstrated a radiation effect on the afferent arc in the graft placed close to the thoracic duct and showed interference with specific graft antigen. The less significant prolongation in the grafts non adjacent to the thoracic duct was felt to be due to general lymphocyte depletion.

HAHN et coll. attempted to prolong renal homograft survival in dogs with intravenous injections of ^{198}Au, but this was unsuccessful. The same substance in colloidal forms has also been infiltrated directly into the lymphatics and similar experiments have been made with ^{206}Bi, ^{111}Ag, ^{90}Y, ^{32}P and ^{131}I tagged ethiodol or antigen. Success has been variable and the method is not practical in clinical use (HUME & WOLF 1967).

F. Irradiation of thymus and spleen. Theoretically, this method should decrease the ICSL population. No benefit has however been found in renal allograft prolongation in numerous studies and the method has been abandoned.

SUMMARY

Organ transplantation and the phenomenon of rejection and its prevention are discussed. The role of radiation is specifically discussed based on animal kidney and heart transplants performed at the Medical College of Virginia.

ZUSAMMENFASSUNG

Organ-Transplantationen und die Erscheinung der Abstossung und deren Verhinderung werden besprochen. Auf die Bedeutung der Bestrahlung wird eingehend mit Hinblick auf Nieren- und Herztransplantationen, die an der Medizinischen Hochschule von Virginia bei Tieren ausgeführt wurden, eingegangen.

RÉSUMÉ

Les auteurs étudient la transplantation d'organe, le phénomène de rejet et sa prévention. Ils étudient le rôle des radiations en se basant sur les greffes de rein et de cœur d'animaux faites au Medical College de Virginie.

REFERENCES

To be given in part II of this paper.