REVIEW ARTICLE

Arterial Homografts

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Arterial homografts, formerly called homografts, came into limited use in the 1940s and 1950s as arterial substitutes. Fresh allografts underwent rapid rejection. Preserved allografts had a longer but still limited clinical life. Allografts demonstrated that arterial replacement was a valid concept and led to the development of synthetic substitutes. Recent renewed interest is based on the need for graft replacements in re-do procedures and in an infected field. Even the best methods of graft procurement and preservation do not preserve normal endothelial and smooth muscle cell functions nor eliminate antigenicity. The biologic and economic costs of immune suppression to obtain a successful allograft for an ischaemic limb are presently unjustifiable. Transplantation between species (xenotransplantation) may be attainable via selective inhibition of the complement system avoiding full immunosuppression now required for organ transplantation. At present allografts may be an acceptable choice for the patient with (1) a critical need for revascularisation and with a life expectancy not exceeding that of the graft, (2) in urgent vascular trauma, and (3) where immunosuppression is contraindicated as in an infected surgical field. Except in most unusual circumstances allografts should not be used for (1) relief of claudication, (2) in the above mid-calf location and (3) anatomic locations where synthetic grafts are superior.

Key Words: Arterial graft; Allograft; Homograft; Transplantation; Immunosuppression; Complement.

Introduction

The first recorded use of arterial and venous homografts was apparently by German surgeons during World War I. Other casualties provided the grafts which invariably thrombosed. Gross et al. in 1948, reported the use of allegedly viable human arterial homografts taken from trauma victims. Contrary to belief based on fibroblast cell cultures these grafts did not retain viability, and at best, may have merely provided a scaffold for ingrowth of host cells, an observation of Guthrie 30 years earlier. In a 1947 review of 12 “viable homografts” inserted in dogs, nine “broke down” and intimal sclerosis and fibrosis occurred in three. Haemorrhage was the cause of death in 75%. Because of their presumed lesser immune response, nonviable (preserved) grafts came to be preferred.

Arterial Homografts (Allografts)

The need for graft replacement of arteriosclerotic vessels was much greater than for correction of congenital and acquired cardiovascular defects. Initially the problem was seen as merely mechanical, replacing a diseased peripheral artery with a normal one for its blood conducting properties. Fresh allografts underwent rapid rejection and dissolution. Preserved grafts were thought to be less so and arterial banks became standard in many vascular centres. Harvesting, sterilising, and preserving grafts utilised formalin, alcohol, glycerine, ethylene oxide, betapropiolactone, high voltage cathode ray irradiation, and freeze drying. Each had its shortcomings. Abundant historical evidence that complex tissues cannot be exchanged between numbers of the same species was ignored. In time preserved grafts displayed their shortcomings as well. Nevertheless, driven by increasing clinical need and lack of a satisfactory substitute, vascular surgeons were quick to use the preserved allograft in the 1950s. In 1952, Dubost et al. inserted a
homograft after resection of an abdominal aortic aneurysm followed almost simultaneously with loss of the fibrillar structure of the elastic lamina of the aortic graft created a localised area of marked reduction of tensile strength. These two events, conversion of the fibrillar structure of the elastic lamina of the wall to an amorphous substance and atheromatous invasion, are the probable starting point of aneurysm formation. 28

Venous Homografts (Allografts)

The belief that venous allografts would function satisfactorily was based on several largely erroneous assumptions:

1. veins were less antigenic than arteries because of their lesser amount of smooth muscle 29,30
2. freezing, prior to transplantation, rendered veins even less antigenic 31 and
3. because of a thinner wall the vein allograft may derive more nutrients from intraluminal flow than arteries, which, being more dependent on vasa vasorum, are more likely to undergo ischaemic degeneration leading to fibrosis and aneurysm formation.

Transplantation antigens were demonstrated on endothelial cells as early as 1971,32 yet their use was continued in the arterial position with 45% 4 year patency rates 32-35. Based on 46 patients, Tice and Zerbino stated in 1972 that “at present vein allografts are safe and satisfactory substitutes when satisfactory autogenous vein is not available;” they also maintained that problems with infection, aneurysmal dilatation, acute necrosis or atherosclerosis were not seen. 34-35. Immunologic factors were considered the major cause of failure of canine allografts (64%) compared to autografts (12.5%), and, whatever the mechanism, Perloff demonstrated that allografts definitely sensitised their canine and rat hosts. 36 In 1975, Williams et al. demonstrated in a canine model that the endothelium was virtually destroyed by day 11 after implantation, medial necrosis was apparent by day 20 and was universal in all allografts by day 40. Smooth muscle was replaced by dense connective tissue, monocytes infiltrated the endothelium penetrating below the internal elastic lamina as in early atherogenesis, and the eventual result was a tube devoid of smooth muscle and consisting largely of collagen. 37

Synthetic Grafts

The arterial allograft experience, although of very limited success, and plagued by cumbersome methods of procurement and preservation, was a major impetus in the development of vascular surgery because it demonstrated that substitutes could work in the arterial system. Essentially biologically inert synthetic materials into which connective tissue could grow from the host and which provided support for a non-thrombogenic surface gave rise to reconstructive vascular surgery. The best of available synthetic tubes perform poorly in regions of low flow and high resistance, in re-do operations increasingly distant from a main conduit vessel, and in areas of infection. All too often autogenous saphenous vein is lacking or of poor quality. The increasing extension of surgical indications into more distal and smaller arterial beds,
the greater complexity of the vascular reconstruction problem, the increase in secondary re-do operations and the presence of infection make a revisit to the venous (and arterial) allograft quite understandable.

**Return of the Allograft**

A number of anecdotal reports recording functioning allografts 10 and more years after insertion, usually of aortoiliac segments, demonstrated that not all allografts were failures. The larger smooth muscle component of muscular arteries such as the femoral and popliteal, compared to the greater elastic content of the iliac and aortic segments rendered the former more antigenic and accounted for their poorer function. The further presumption was that elastic elements were less immunogenic than muscular elements. These observations as to wall composition and greater durability of elastic implants are correct. The assumptions concerning degrees of antigenicity are just that for there seem to be formal confirming data. The antigenicity of muscular and elastic arteries must be greatly reduced by the cytotoxicity of sterilisation and preservation. It is surprising that so little analysis has been done on the immune aspects of the artery and the possibility of immunosuppressive treatment after implantation given the potential usefulness of the technology.

**Cryopreservation**

Although many laboratory and clinical studies over many years, have examined the results of cryopreservation, irradiation and immunosuppressive therapy, and most were modestly optimistic, the clinical studies were uncontrolled, non-randomised, and of short follow-up. Boren and Moore concluded that cryopreservation of canine artery segments in liquid nitrogen and 15% dimethyl sulphoxide (DSMO), an oxygen radical scavenger, was an excellent method for preserving and storing fresh allograft arteries for use as a small vessel replacement. Careful analysis yields the realisation that, in reality, preserved grafts are superior to fresh grafts, an observation already accepted in clinical practice. Abbott’s group reported that short-term freezing of canine jugular venous allografts preserved the important functional elastic properties of veins independent of DSMO. Giordano combined irradiation with freezing of canine external jugular veins placed in the femoral position and compared their function with autografts at 6 months. Eighty-three percent of the autografts and 72% of the prepared allografts were patent. They suggested that the recipient’s immunologic reaction to the graft did not affect patency and further suggested that irradiation may have significantly reduced the immunologic potential of the graft. These were speculations unsupported by objective, controlled data and weaken their concluding statement that systemic suppression of the
recipient's immune response may not be necessary if frozen, irradiated allografts are used for arterial or venous replacement. Numerous other studies with variations in technique reported modestly favourable results but they all suffered from non-randomisation, small series, limited follow-up periods and sparse histologic observations. A literature review of venous allografts revealed a 1 year patency rate of approximately 50%.

Despite many attractive aspects of studies of experimental saphenous allograft clinical usage had a high failure rate. Evidence continued to identify failure as initiated by immunologically induced thrombosis as a consequence of damaged endothelium. Better patency was achieved with frozen than with fresh allografts. Freezing was a deliberate attempt to destroy cellular elements and thereby reduce antigenicity as well as to facilitate storage.

Attempts to improve allograft performance include histocompatibility matching of the vein donor and allograft recipient, graft recipient immunosuppression, blood group matching and pretreatment of grafts with immunosuppressive agents. Cryopreservation, in contrast to simple freezing and frozen storage techniques, is intended to preserve some cell functions by careful control of rate of cooling and prevention of ice nucleation. It has been labelled cryoprotection based on an observation that glycerol exerted a protective effect on frozen and thawed spermatozoa, and that freeze damage to living cells could be prevented by DMSO, especially when combined with chondroitin sulphate. Barner et al. appear to have applied it to vascular surgery in 1966.

Fig. 4. Chondroitin sulphate promotes endothelial cell survival in cryopreserved veins. Limiting dilution assay results comparing survival of clonogenic endothelial cells in canine saphenous veins cryopreserved with 10% dimethyl sulfoxide (DMSO) alone or in combination with 2.5% chondroitin sulphate (CS). Results of experiments in which chondroitin sulphate was employed in all stages of vein processing except during cryopreservation in the presence of dimethyl sulfoxide, zero cryoprotectant controls, and untreated controls are also presented. NS = no significant difference. * = statistical significance using a paired t-test at the p < 0.05 confidence level. From: Brockbank KGM. Effects of Cryopreservation upon Venous Functions. In: Brockbank KGM, ed. Principles of autologous, allogeneic, and cryopreserved venous transplantation. Austin, TX: RG Landes, 1995. With permission.
quantity to sustain contraction. Evidence of rejection was observed in all allografts. Cyclosporine, for immune suppression and aspirin for platelet control, were used selectively. Both freshly harvested and cryopreserved allografts showed signs of rejection even with cyclosporin immunosuppression. Even with evidence of rejection grafts remained patent, but patency was not examined beyond 30 days of implantation. In summary, despite extensive experimental study and scattered encouraging clinical reports, cryopreserved vein grafts in human arterial reconstructions have had only limited success. Refinements in graft harvesting and processing, multiple cryoprotectants, and partial immunosuppression have not entirely solved the problem of vascular allografts. Immunologic response of the host to the graft appears to be a continuing and major problem.

**Immunosuppression**

The successful use of both venous and arterial allografts in both the venous and arterial circulations requires that they be considered and treated as true organ transplants as has been the case for kidney, liver and other organs for decades. To continue to attempt to achieve success with partial solutions, valuable as they have been in defining the scope of the problem, is to risk waste of limited research resources and time. Under special and limited conditions the vascular allograft can be used successfully without conventional transplantation immune suppression therapy. These special situations include, but probably are not limited to: (1) the individual with a severely ischaemic leg and a life expectancy not exceeding the functional life of the allograft, (2) emergent replacement of a short segment of a major vessel lost to trauma or sacrificed to malignancy, (3) when immunosuppression therapy for other organ transplantation immune suppression therapy for kidney transplants who subsequently required peripheral arterial revascularisation.

To expand on the first point: cell surface structures involved in allograft rejection are identified variously as transplantation antigens, endothelial cell surface antigens, histocompatibility antigens, and human leukocyte antigens (HLAs). These are encoded in a genomic location known as the major histocompatibility complex (MLC). Arterial and venous allografts obtained at random from unrelated donors may very likely differ substantially from the recipient's HLA antigens and thus can engender rejection. Class I antigens (HLA A, B and C loci) recognise "killer" T lymphocytes. These T cells mediate the damage to the allografts and constitute the effector limb of the cellular immune response. The afferent limb of cellular immunity is furnished by the interaction of helper T lymphocytes with Class II molecules resulting in stimulation of the activity of the killer T cells. Endothelial and smooth muscle cells, which might be present on cryopreserved arterial allografts, can also express Class II antigens. To initiate the immune response arterial allografts must contain viable cells that have survived the preservation and storage processes, must retain, after thawing, the ability to express Class II HLA, and finally, must continue to bear Class I HLA in order to permit recognition as targets by the effector killer T Cells.

In 1993, we reported that cryopreservation of arterial allografts did not eradicate smooth muscle cells. At the time of surgery we cultured aortic allograft-derived smooth muscle cells from unused portions of antibiotically treated cryopreserved allografts obtained from the major commercial supplier at that time. These cells reacted within a human monoclonal antibody that recognises smooth muscle actin isoforms, thus identifying them as smooth muscle cells. Under basal conditions these cells contained messenger ribonucleic acid (mRNA) for Class I HLAs detected by northern blotting and expressed Class I HLA on their surface as measured by enzyme-linked immunoassay (ELISA). Interferon gamma, a product of activated T lymphocytes, not only increased expression of Class I HLAs by smooth muscle cells, but induced Class II human leukocyte mRNA and elevated surface expression from 22 to 819 ELISA units. Thus, these studies demonstrated that cryopreserved aortic allografts contain viable smooth muscle cells capable of expressing major histocompatibility antigen that might render them immunogenic and susceptible to rejection by the recipient's immune system.

The basis for the second observation is the demonstration by daGama et al. of satisfactory performance of arterial allografts in humans under immunosuppression therapy for kidney transplants who subsequently required peripheral arterial revascularisation.
Arterial Homografts

Thirteen kidney transplant patients on maintenance azathioprine, cyclosporine and prednisone received 16 ABO-compatible arterial allografts. Twelve femorofemoral, popliteal and femoroperoneal bypass grafts were inserted plus one aortoiliac, one iliofemoral and two iliofemorals. The arteries were retrieved from a cerebral death donor and stored in liquid nitrogen vapor. Patients were monitored for up to 45 months with a mean of 20 months. Graft tolerance was good; there were no signs or symptoms of acute graft rejection, and there was no perturbation of the immunologic tolerance of the transplanted kidney. During the follow-up period two grafts occluded, but on the basis of histologic studies, it was not possible to relate the occlusion to rejection.

Immunosuppression, however, does not always eliminate rejection. When successful, it reduces the intensity and duration of the response, but the threat is always present. The biologic cost to the immuno-suppressed patient and the economic cost to society seem out of proportion to the benefit derived, if the indication is a severely ischemic limb. A life threatening situation may improve the cost-benefit ratio. A candidate for a peripheral arterial reconstruction who is already receiving immunotherapy is a special situation.

Complement

Morphologic evaluation of allografts harvested as late as 5 weeks after implantation has revealed heavy infiltration with polymorphonuclear leukocytes (PMN). Activated PMNs are known to damage endothelial cells in a variety of disease conditions such as immune vasculitis. In 1984, in the course of studying the feasibility of endothelial cell seeding of synthetic grafts, we noted the occasional appearance of inflammatory cells in the newly formed intima despite an apparently satisfactory re-endothelialized graft surface. Subsequently we demonstrated activation of the complement system via both the classical and the alternative pathways by what formerly had been assumed to be a biologically inert synthetic graft. This is probably the first evidence that complement is a participant in arterial injury healing. It now appears that complement occupies a critical role in several aspects of the rejection phenomenon. Inhibition of the complement cascade may lead to improved management of the transplant patient. Ignoring for this discussion the hyper-acute rejection which can begin within minutes of reperfusion, and chronic rejection, such as cardiac transplant atherosclerosis and lung transplant obliterator bronchiolitis, acute or vascular rejection is generally thought to be due to recipient T cell recognition of disparate major and minor histocompatibility antigens by either a "direct pathway" or an "indirect pathway". "Direct allo-recognition" occurs when recipient T cells respond to intact allo-MHC molecules on the surface of allogeneic cells. "Indirect allo-recognition" occurs when allo-MHC molecules are processed by recipient antigen presenting cells (APC). Complement receptor 1 (CR1, CD35) is an example of a natural inhibitor of the complement cascade. It has now been shown that a recombinant soluble version of complement receptor 1 (sCR1) inhibits both the classical and the alternative pathways of the complement cascade in vitro, and is effective in prolonging organ function in several in vivo transplant models.

An additional potential way of blocking the T lymphocytes is via the recently discovered costimulatory molecule B7-2. Two signals are required for T cell activation:

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Fig. 6. Granulocyte aggregometry tracings showing greater PMN aggregation (as measured by large increase in light transmission ($\Delta T$)) for plasma exposed to knitted Dacron than to PTFE. Zymosan-activated plasma (AZAP) (4 mg zymosan/1 ml plasma) reflects maximal PMN aggregation by known potent activator of complement system. From: Shepard AD, Gelfand JA, Callow AD. Complement activation by synthetic vascular prostheses. J Vasc Surg 1984; 1: 829-838. With permission.

Fig. 7. A simplified schema of the complement system depicting the classical and alternative pathways and the possibility of dual activation by vascular grafts. From: Shepard AD, Gelfand JA, Callow AD. Complement activation by synthetic vascular prostheses. J Vasc Surg 1984; 1: 829-838. With permission.
(1) the specific antigen recognised by the T cell and
(2) a costimulatory signal located on the surface of the antigen presenting cell activating via receptors CD28 and CTLA4 on the cell. Absence of or blocking the co-stimulatory signal places the T cell in a state of anergy, in effect, shutting off the T cell specific for a given antigen. B7-2 is claimed to be the major player in activating T cells. CTLA4-Ig is a specific blocker of B7. It thus has the potential to block the T cell rejection response to the transplant.73

Eventually xenotransplantation of arteries and veins may become possible.

Summary

The problems of unsatisfactory small calibre synthetic grafts, re-do vascular operations, and infected vascular grafts have all contributed to the recently renewed interest in the vascular homograft, more correctly called, the allograft. The flaws of the allograft during the 1950s and 1960s have not been solved despite enormous improvements in harvesting, preservation and storage. Cryoprotectants have reduced the severity and rapidity of onset of allograft failure but not eliminated them. Immunosuppression therapy has reduced the failure rate somewhat more. Best results with allografts are seen in individuals who require major arterial restorative surgery and are under full immunosuppression because of a previously placed organ transplant. Full immunosuppression is probably too high a biologic price to pay to relieve an ischaemic limb, but limited suppression via inhibition of the complement system may not be. Continuing investigation may yield the answer. Until then allografts appear to be a useful choice for (1) the individual with a real need for revascularisation and who is burdened with a limited life expectancy not exceeding the functional life of the allograft, (2) urgent replacement of a major vessel damaged by trauma, and (3) in situations where immunosuppression is contraindicated as in an infected surgical field. The allograft probably should not be used (1) simply for the relief of intermittent claudication, (2) in the above mid-calf location, and (3) in other anatomic locations where synthetic grafts perform better.

Fig. 8. Scanning electron micrograph of surface of knitted Dacron graft after 2.5 h of flow in an \textit{ex-vivo} shunt circuit. Note the adherent PMN membrane ruffling and pseudopod formation which indicate an activated state. (Original magnification × 13 500). From Shepard AD, Geland JA, Callow AD. Complement activation by synthetic vascular prostheses. \textit{J Vasc Surg} 1984; 1: 829-838. With permission.

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