Fetal Allograft Survival in Immunocompetent Recipients Is Age Dependent and Organ Specific

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This study explores whether fetal allograft survival is age dependent and organ specific. Fetal rat tissue (renal, gonadal, hepatic) from the third trimester of gestation (days 15–21) was transplanted into 306 outbred adult rats for 10–30 days. Grafts were studied by morphometric and histologic analysis. Ten days after implantation, renal tissue (N = 75) from late gestation (days 19–21) showed no increase in size. In contrast, 17-day fetal grafts (N = 20) grew 6.8 ± 3.4 times,* while 15-day fetal grafts (N = 28) grew 17.5 ± 6.1* times. (The symbol "*" indicates $p < 0.05$, compared to original size.) Twenty days after implantation, these 15-day fetal grafts (N = 20) grew 48.8 ± 17.7* times. Ten days after grafting, the younger fetal tissue showed excellent maturation of renal elements and no sign of rejection; older fetal grafts had poor renal architecture and a dense lymphocytic infiltrate. The 15-day fetal gonadal tissue (N = 18) showed a moderate 10.6 ± 3.2* increase in size while the 15-day hepatic grafts (N = 16) were regularly rejected within 10 days. Selected fetal allografts from early in the third trimester can not only survive but can grow and mature in an immunocompetent recipient. This fetal graft growth appears to be both age dependent and organ specific. The use of fetal organs may broaden the potential pool for transplantation. However, further studies are needed to define the ontogeny of graft acceptance.

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IN AN ATTEMPT to broaden the pool of available organs for transplantation and to understand better the mechanisms of graft rejection, this laboratory has embarked on a program to investigate the antigenicity of fetal donor tissue. It is well known that the immunocompetent adult promptly rejects allogeneic adult tissue soon after transplantation.[1] Studies from other laboratories using fetal small intestinal or pancreatic tissue from late in gestation showed prompt rejection in allogeneic adult recipients.[2,3] In contrast, we have demonstrated that fetal kidney and adrenal tissue from early in the third trimester survived and grew when transplanted into adult allogeneic recipients.[4] These seemingly divergent results prompted us to ask whether fetal graft growth might be both age dependent and organ specific. To this end, we varied the ontogeny and the organ of the fetal grafts that were implanted into adult allogeneic animals.

Materials and Methods

Animals

Outbred Sprague–Dawley rats were obtained from Holtzman Laboratories, Madison, Wisconsin. All recipient animals were adult females over 10 weeks of age and weighed 225–250 g. Timed pregnant mothers were shipped between 11 and 13 days of pregnancy. A total of 306 animals received grafts.

Graft Tissue

The graft tissue consisted of renal tissue harvested from adults (Group I) or fetal tissue: renal (Group II), gonadal (Group III), or hepatic (Group IV). Adult donor rats underwent cervical dislocation, and their kidneys were rapidly excised, placed in Dulbecco's Modified Eagle's Medium at 4C, and divided under a dissecting microscope into fragments less than 1 mm in diameter. Fetuses were delivered by cesarean section from timed pregnant mothers on the fifteenth to twenty-first day of gestation. The liver, kidneys, and gonadal tissue from successive days in gestation were excised, placed in 4C culture medium, and

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also divided into fragments less than 1 mm in diameter. Selected fetal livers were harvested as early as day 13 of gestation and minced as above.

Grafting Method

The subrenal capsule grafting technique was described by Bogden[5] for tumor implantation in mice and later adapted by this laboratory for implantation of fetal tissue.[4,6] Adult recipient rats were anesthetized with 0.9 ml of 12% chloral hydrate by intraperitoneal injection and their left flanks cleaned with alcohol and shaved. A 2 cm flank incision was made, and the left kidney was retracted out of the wound. The renal capsule was gently elevated and incised with a #11 scalpel blade, and the graft tissue, mounted on a 16-gauge trocar, was slipped under the renal capsule over the renal parenchyma. A 6-0 Prolene® suture was then placed adjacent to the graft tissue under the renal capsule to serve as a marker. In each animal, two grafts were placed under the renal capsule in different locations. We measured the long (L) and short (W) axes of the grafts after implantation at 10× magnification and a standard focal distance, using a microscope equipped with an eyepiece calibrated so that 10 ocular micrometer units equaled 1 mm. The kidney was then replaced in its normal position and the flank wound closed with sutures and skin staples. The animals were returned to their cages and, after awakening, were fed water and rat chow.

Graft Takedown, Morphometric, and Histologic Assessment

The animals receiving implants were killed by cervical dislocation between 10 and 30 days after transplantation; the left kidney was excised, placed in 10% formalin, and long and short axes of the graft again measured. We calculated the graft size ratio by multiplying L × W, both for the original graft (Pre-size) and the graft at the time of takedown (Post-size). The change in graft size, or graft size ratio (GSR), was calculated as:

$$\frac{\text{Post-size}}{\text{Pre-size}} = \text{Graft Size Ratio (GSR)},$$

with GSR > 1: a graft that had increased in size; GSR = 1: no change in size; GSR < 1: a graft that had decreased in size; and GSR = 0: only scar found (rejected).

The average of the GSR of the two grafts in each kidney was used for analysis. The grafts were then excised from the host kidney, placed in agar, and fixed for 48 hours in Mirsky's solution. They were then processed through a series of alcohol dehydration steps, cleared in xylene, fixed in paraffin, and stained with hematoxylin and eosin.

Fetal renal grafts were assessed for maturation of histologic architecture. We first constructed a library of *in vivo* renal histology for tissue from days 15 to 21 of gestation and from days 1 to 7 *post partum,* and for adult tissue to which we could compare architectural and histological development after implantation. We then assessed the degree of maturation of fetal renal architecture and development at the time of graft takedown 10, 20, and 30 days after transplantation. The histology of each graft was rated on a semiquantitative scale of 1 (best) to 5 (worst). A graft that had excellent glomerular and tubular development was rated a "1"; a "3" was assigned to a graft with a modest degree of maturation of tubules and glomeruli; while a "5" indicated the poorest histology where no renal elements could be identified. In a similar manner, the degree of lymphocytic infiltrate as a measure of graft rejection was assessed, again using a 1 to 5 scale, with "1" being no infiltrate, or no histologic signs of graft rejection, and "5" being a very dense infiltrate, or a completely rejected graft. Grades of "2," "3," and "4" were given for grafts that showed evidence of progressive rejection or increasing lymphocytic infiltrate. These morphologic analyses provided the bases for two bioassays. All statistical analyses of graft size ratio, changes in graft architecture, or change in lymphocytic infiltrate were expressed as mean ± standard deviation and were compared by Student's t-test.

Results

Group I (Adult Renal → Adult)

A total of 12 rats received adult renal allografts. The recipients were sacrificed 10 days after grafting, and at that time all grafts were totally rejected.* In the majority of cases, only a small scar was present in the area that had received the graft.

Group II (Fetal Renal → Adult)

One hundred seventy animals received grafts of fetal renal tissue from days 15 to 21 of gestation, and the recipients were killed 10 days after transplantation (Fig. 1). Fifteen-day fetal renal grafts (N = 28) increased 17.5 ± 6.1 times,* and the 16-day fetal grafts (N = 25) showed a 14.7 ± 6.6 increase.* A representative 15-day fetal graft 10 days after implantation in Figure 2 shows good growth and neovascularization from the host kidney. The 17-day fetal grafts (N = 20) showed a modest 6.8 ± 3.4 increase in size,* while the 18-day fetal grafts (N = 22) increased only 3.8 ± 2.9* times. In contrast, the fetal renal tissue from later in gestation showed little growth: 19-day fetal tissue (N = 21) grew only 2.1 ± 2.3 times, 20-day fetal grafts (N = 26) grew 1.8 ± 1.8 times, and 21-day fetal grafts (N = 28) increased 1.9 ± 2.6 times.

* $p < 0.05$ compared to the original size.

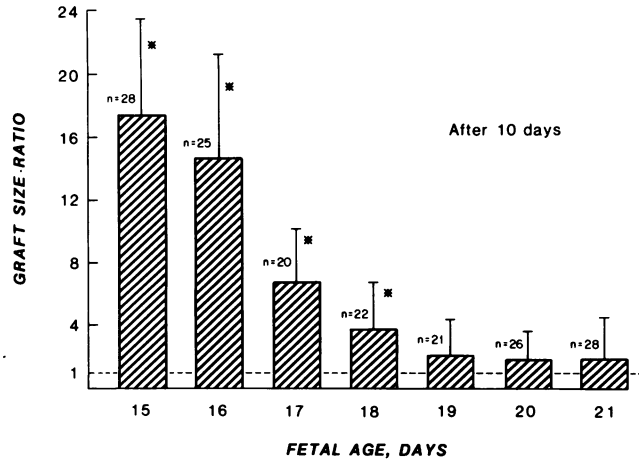


FIG. 1. Increase in size ($\bar{x} \pm$ S.D.) of fetal renal tissue implanted in the adult subrenal capsule. Grafts from 15 and 16 days of gestation grew well, while older fetal grafts grew poorly. n = number of animals; *p < 0.05, compared to original graft size.

Fifteen-day fetal renal grafts implanted into 63 other recipients were examined between 15 and 30 days later. After 15 days, the grafts in 20 animals showed a significant (28.9 ± 9.1†-fold) increase in size when compared to the grafts implanted for only 10 days. Grafts in another 20 recipients killed 20 days after transplantation increased in size 48.8 ± 17.7† times; those in 23 animals implanted for 30 days demonstrated a 48.3 ± 23.2 increase† in fetal graft growth.

Histologic Bioassay

Semiquantitative analysis of renal architecture (1 = excellent; 5 = poorest) and determination of the degree of lymphocytic infiltrate (1 = best, least infiltrate; 5 = worst, maximum infiltrate) were carried out on grafts transplanted into 154 animals, which were killed 10 days after grafting. The 15-day gestational age grafts had a proliferation of glomeruli and tubules, and blood vessels were present throughout the graft (Fig. 3A). These grafts 10 days after implantation appeared comparable in development to a 20–21-day fetal kidney. There was a marked contrast in the development of renal elements (Fig. 4) between the 15-day [(N = 19) 1.73 ± 0.71] and 16-day fetal grafts [(N = 23) 2.07 ± 0.99] when compared to the grading of the grafts from later in gestation, *i.e.*, days 20 and 21 [(N = 54) 4.79 ± 0.41] ($p < 0.05$). Likewise, the semiquantitative assessment of lymphocytic infiltrate (Fig. 5) for the 15-day fetal grafts [(N = 19) 1.85 ± 0.84] and 16-day fetal grafts [(N = 23) 2.44 ± 1.12] was reasonably good, while the grading for the older 20- and 21-day fetal grafts (N = 54), also implanted for 10 days, was poor (4.89 ± 0.29; $p < 0.05$) (Fig. 3B).

Histologic assessment was also carried out in 19 animals that had received 15-day fetal renal grafts 20 days previously. In contrast, these larger grafts had a significantly poorer architectural grading, 3.54 ± .86,‡ and a more pronounced degree of lymphocytic infiltrate, 3.72 ± .89,‡ compared to the grafts implanted for only 10 days (Fig. 6). At 30 days after transplantation in 19 animals, the histologic changes were worse still, although growth was considerable. Architectural grading was 4.6 ± 0.7,‡ and there was a heavy lymphocytic infiltrate graded 4.7 ± 0.5.‡

Group III (Fetal Gonad → Adult)

Fifteen-day fetal gonadal tissue was implanted into 45 adult recipients. Ten days after transplantation, these grafts in 18 animals showed a 10.6 ± 3.2* times increase in size. By 20 days, in 19 grafted animals, the implants had grown 31.2 ± 12.6 times.*,† After 30 days the grafts in eight recipients had increased in size 40.7 ± 20.9*,† times (Fig. 7). Compared to the progressive worsening of the architecture and infiltrate of the renal grafts 20 and 30 days after implantation, the 15-day fetal gonadal grafts had less evidence of rejection and also exhibited maintenance of relatively good gonadal architecture 20 and 30 days after implantation.

Group IV (Fetal Hepatic → Adult)

In contrast to the growth seen with 15-day fetal renal or gonadal tissue, the 15-day fetal hepatic tissue transplanted into 16 recipients and analyzed 10 days later showed only a 1.9 ± 3.3-fold increase in size ($p <$ NS, compared to original size). In the few grafts available for histologic examination, only a small number of viable fetal hepatic cells were present. In all other cases, only scar tissue was present. Several 13-day fetal grafts also were implanted for 10 days; these too stimulated an intense infiltrative response within 10 days.

Discussion

It is well known that when adult allogeneic tissue is transplanted into an immunocompetent host, rejection occurs promptly. The major research efforts in transplantation over the past 2 decades, which centered on identifying the "degree of foreignness" of the donor (tissue typing, HLA) and designing methods of immunosuppressing the recipient, have produced considerable progress. Immunosuppression of the host, however, carries significant risks for the development of infection, or longitudinally, of malignancy. The paucity of organs available for transplantation can result in death or increased morbidity for the recipient. The limitations of both recipient

† $p < 0.05$, compared to graft size 10 days after implantation.

‡ $p < 0.05$, compared to 10 days after grafting.

treatment and organ availability prompted this laboratory to study methods of modulating donor tissue, both to better understand the mechanisms of graft rejection and to broaden the pool of potential organs for transplantation. Our hypothesis was that by varying the ontogeny of the donor tissue we could identify a time in development at which selected grafts might survive and grow. Tissue from different fetal organs were also used to test the hypothesis that fetal graft growth might be not only age dependent, but also organ specific. Further study of the conditions favoring graft survival of specific organs at particular stages of gestation could elucidate methods of modulation either to discourage or to encourage rejection. The former would be clinically applicable to transplantation and the latter to control of neoplasia.

The rat was chosen because fetuses were available in large numbers from accurately timed pregnancies. In each experimental group, outbred immunocompetent adult female rats were used as recipients. Selected studies, carried out using male recipients, indicated no difference in graft tolerance based on host gender. In group I (adult renal graft → adult recipient) no viable graft was present by 10 days after implantation. This confirmed the fact that the host was immunocompetent and could reject adult allografts.

We have shown previously that selected fetal tissue from early in the third trimester of gestation could survive and grow under the same conditions.[4] The present study detailed each day of gestation from the time when the kidney was easily identified at 15 days until 21 days, the last day of gestation in the rat. Fifteen-day fetal renal grafts showed a marked increase in size after only 10 days of implantation. When fetal renal grafts from progressively later periods in gestation were implanted for the same length of time, there was a proportional decrease in graft growth as older renal tissue was used. The fetal grafts from day 19 of gestation and later were statistically no different in size than at the time of implantation.

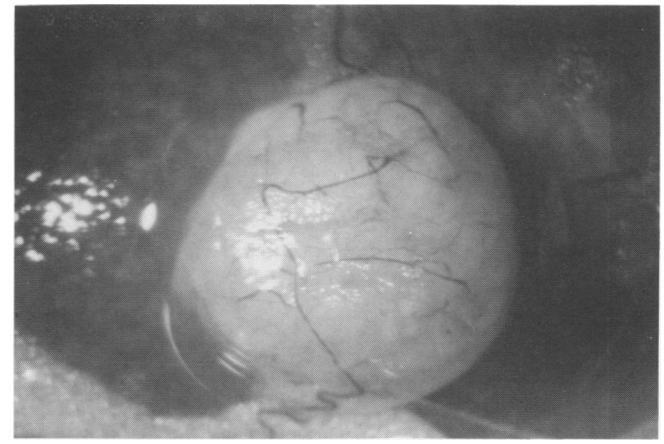
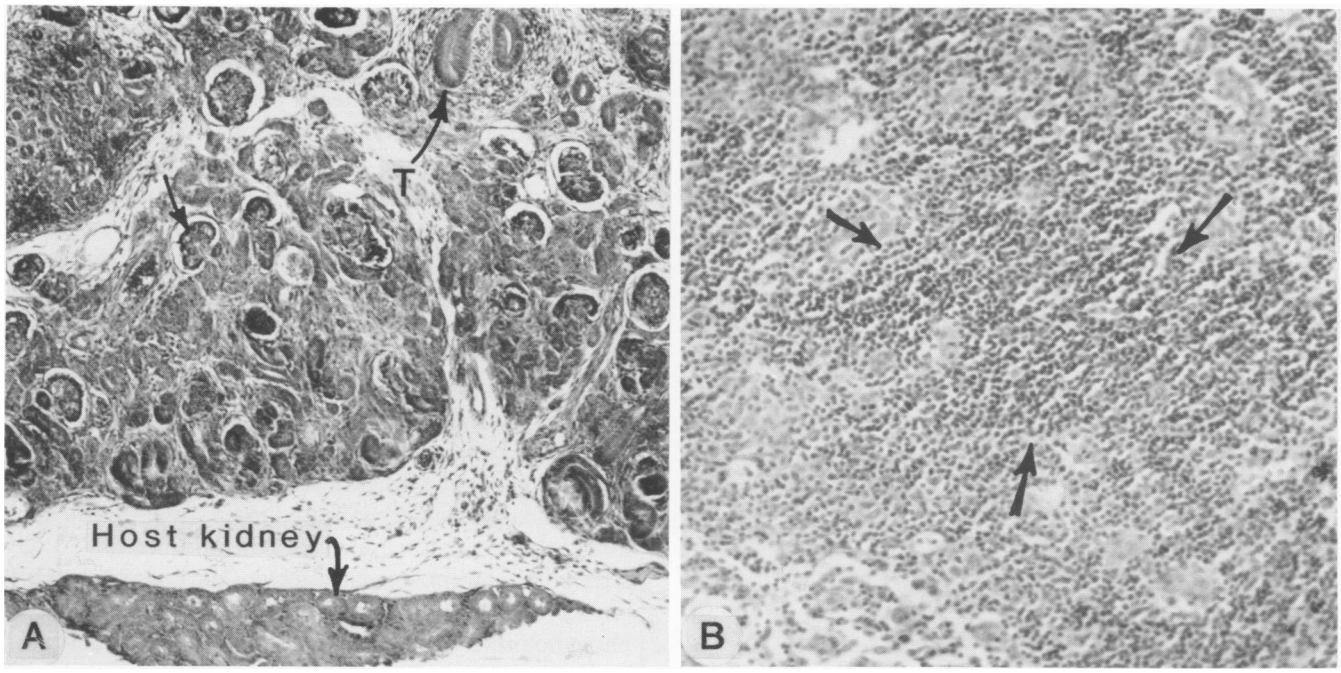


FIG. 2. A 15-day fetal renal graft 10 days after implantation. This plump and well-vascularized implant has increased in size seventeen-fold.



FIGS. 3A and B. Histology of fetal renal tissue 10 days after implantation. *A*. Fifteen-day fetal graft: note the proliferation of glomeruli (G) and tubules (T), but no infiltrate. (150×). *B*. Twenty-day fetal graft: few renal elements present, and a heavy lymphocytic infiltrate (arrows) is present (150×).

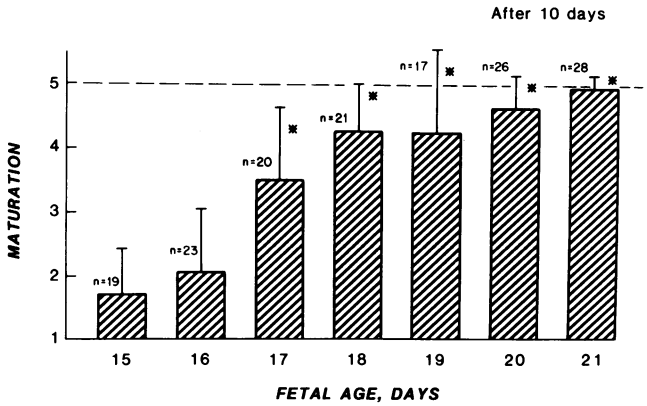


FIG. 4. Bioassay of architecture of fetal renal histology 10 days after implantation. Early fetal grafts show good maturation of renal elements. Grafts from later in gestation have fewer and finally no recognizable renal elements; scale: 1 = best, 5 = worst; $\bar{x} \pm$ S.D.; *p < 0.05 *vs.* 15-day fetal grafts.

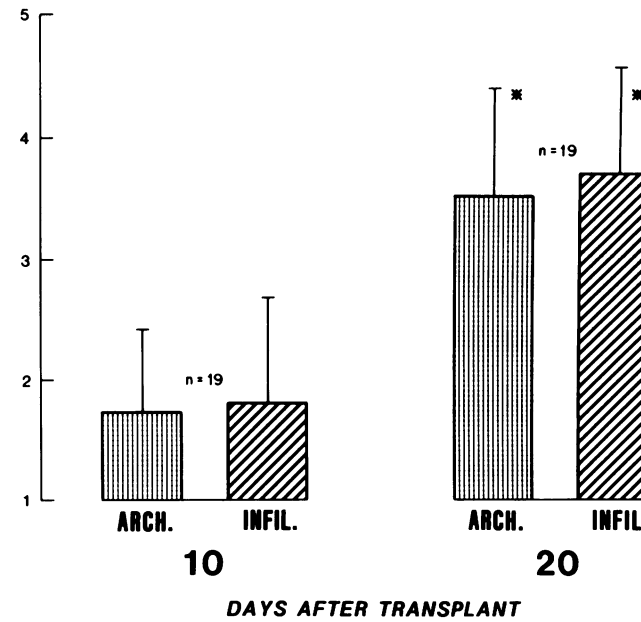


FIG. 6. Comparison of 15-day fetal renal maturation and infiltrate 10 and 20 days after implantation. While longer periods of implantation allow greater graft growth, there was a worsening in architecture and an increased lymphocytic infiltrate. $\bar{x} \pm$ S.D.; *p < 0.05 *vs.* 10 days after implantation.

After a significant fetal renal growth with the earlier gestational age grafts and progressively less growth later in fetal ontogeny were documented, histologic changes were studied in each successive day. The 15-day fetal kidney at the time of implantation had a limited number of renal elements present and a large amount of undifferentiated mesenchymal tissue; 10 days after grafting, the renal elements matured, showing an increase in glomeruli and tubules, and mesenchymal tissue diminished. We speculate that the lag in histologic development between the 15-day fetal grafts implanted for 10 days and comparable fetal kidneys allowed to grow normally *in situ* may be due to the 2–3 days' time required for neovascularization to occur. There was little or no lymphocytic infiltrate present, indicating little evidence of rejection. When fetal renal tissue from later periods in gestation was used, progressively less maturation occurred in inverse proportion to the age of the implanted graft, and a proportionately heavier degree of lymphocytic infiltrate was evident.

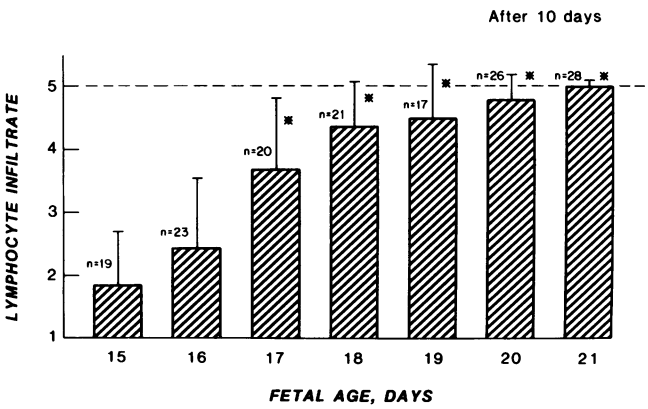


FIG. 5. Bioassay of lymphocytic infiltrate in fetal renal grafts 10 days after implantation. Note a progressive worsening of the infiltrate with the use of older fetal age grafts. Scale 1 = least, 5 = maximal; $\bar{x} \pm$ S.D.; *p < 0.05 *vs.* 15-day fetal grafts.

Having identified the tissue from early in the third trimester as that which grew best, we then, in selected studies, lengthened the time after implantation from 10 to 30 days. These data showed that the grafts increased in size up to 48 times by 20 days and 30 days after implantation. When 15-day fetal renal grafts were inspected histologically at 20 days after implantation, however, there were significant decline in renal architecture and a moderately severe lymphocytic infiltrate. By 30 days after implantation these grafts had little recognizable renal elements remaining and the lymphocytic infiltrate was overwhelming, indicating that the advantage conferred on the younger grafts during the first 10 days after implantation was not permanent. However, the significant observation of early graft survival remains.

We next investigated whether the growth of grafts from earlier in ontogeny might be organ specific. Similar studies with 15-day fetal gonadal tissue again demonstrated privileged growth. The longitudinal growth characteristics of the gonadal tissue also appeared to be similar to the renal tissue; there was continued growth up until 20–30 days after implantation. The histology of the gonadal tissue 10 days after transplantation showed little evidence of a lymphocytic infiltrate. Preliminary studies in our laboratory indicated that gonadal tissue implanted for 20 days in contrast showed little evidence of rejection of germinal tissue, while adjacent fetal mesonephric tissue was

promptly rejected (unpublished data). These and other studies indicating that testicular grafts from older embryos and neonates are not rejected will be the subject of a later report since sex differences in both donor gonadal tissue and recipient require further elucidation. In marked contrast to the excellent growth of fetal renal and gonadal grafts, the fetal hepatic grafts from early in the third trimester of gestation showed essentially no growth. The majority of the grafts were merely scars by 10 days after implantation, and those several that had increased in size had on microscopic examination few viable hepatic cells present. These results demonstrate a wide variation in the ability of donor fetal organs to survive transplantation without immunosuppression. The privilege conferred on some, however, is sufficient to warrant investigation of the mechanisms involved, for eventual application to methods of potential donor modulation.

The concept of using fetal organs for transplantation is not a new one. Experiments using fetal small intestine in a syngeneic model have documented functional absorption from the gut lumen and peristalsis.[7] However, when allogeneic transplants were attempted using 18–20 day fetal rat small intestine, prompt rejection occurred.[2,8] Similarly, Brown reported success with transplanting syngeneic rat fetal pancreas from days 16 and 17 of gestation, while pancreatic tissue from late in gestation showed high failure rates.[9] In contrast, early gestational age fetal pancreatic grafts transplanted into allogeneic recipients were promptly rejected within 7 days.[3] Our findings with fetal allogeneic hepatic transplants corroborated these results.

The results we report with fetal renal and gonadal grafts, in contradistinction, indicate that some fetal organs can not only survive, but grow, and mature histologically when implanted into allogeneic recipients. Thus the success of fetal allogeneic transplantation varies in part with the choice of donor organs. Patthey and Edidin[10] showed that whole embryonic congenic murine grafts differing at only one H-2 locus stimulated an intense infiltrate from as early as the seventh day of gestation, indicating that antigenic expression occurs very early in embryonic development. The seminal work of Simmons and Russell, which pointed to the protective role of trophoblastic tissue in preventing rejection of the fetus by the mother, again demonstrated that murine embryonic tissue from as early as day 7½ of gestation was rejected when transplanted into adult allogeneic mice.[11] Using a mixed hemadsorption assay, Kirkwood and Billington showed that the mouse H-2 antigen is serologically detectable from days 11 and 12 onwards.[12] However, it is not expressed at the same time throughout the embryo, *i.e.*, cells from fetal skin, lung, and forelimb bud expressed H-2 antigen by midgestation, while cells from fetal kidney and gonad had little if any detectable antigen through the end of the second trimester of gestation.

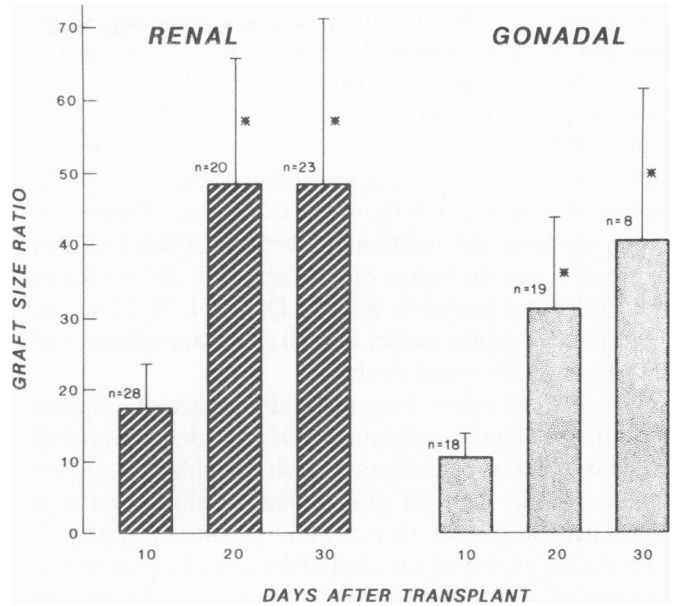


FIG. 7. Increase in size ($\bar{x} \pm$ S.D.) of fetal renal and gonadal grafts at 10, 20, and 30 days after implantation. *$p < 0.05$ *vs.* size at 10 days.

The organ and timing differences—yet ultimate ubiquitousness of rejection of most fetal organs—have focused our attention on the Class I surface antigen as the final common denominator of graft rejection. The compelling appeal of a study of this surface antigen is that an understanding of the role it plays in the favored status of some fetal organs may have some application, on the one hand, to controlling graft rejection or, on the other, by enhancement, to controlling the growth of neoplastic cells.[13] It is well recognized that Class I antigens are a major factor responsible for transplantation rejection and cell-mediated immunity against viral infections and tumors.[14] In the neonate and the adult, Class I surface antigens are present on all cells, and, for this reason, these molecules are often referred to as transplantation antigens.[15,16] Ozato et al. demonstrated nonsimultaneous fetal organ expression of the Class I antigen protein.[17] In addition, they measured Class I antigen mRNA, which was first detected in some organs as early as day 9 of gestation and remained at low levels through days 13–16 of gestation. Thus, it is probable that Class I gene expression is developmentally regulated for each specific embryonic organ.

The murine Class I molecules are encoded by K, D, and L gene clusters of the major histocompatibility complex (H-2),[16,18] which, *via* mRNA, direct formation of a polypeptide, which subsequently becomes membrane bound and glycosylated.[19] The specific molecules consist of a small intracellular domain, a transmembrane hydrophobic portion, and a much larger extracellular region of three external domains associated with $beta_2$ microglobulin.[15] The lack of immunologic response seen with some

fetal tissues may be due to an insufficiency in any of the processing steps described above. mRNA may not be transcribed from genomic or complementary DNA; surface antigenic protein may not be adequately translated from mRNA; or surface antigen may not be biologically active because of incomplete protein formation, configurational changes, or delay of glycosylation.[20] We are currently studying the relationship between Class I antigen expression and the tempo of graft rejection, by measuring both mRNA expression with K, D, and L H-2 loci and $beta_2$ microglobulin probes, as well as surface protein with immunohistochemical probes.

Growth factors are present in abundance and appear essential for fetal development. Of considerable interest is the role that growth factors, such as epidermal growth factor, platelet derived growth factor, alpha and beta transforming growth factor, and insulin, may play in stimulating growth of the grafted tissue and/or conferring a degree of immunotolerance for the graft. We will attempt to correlate the presence of growth factors, the successful transplantation of the fetal grafts, and the interrelationship of these with the presence of Class I antigen mRNA.

Recognition by the host of the Class I antigen appears to be key to the rejection phenomenon. Several authors have shown that certain normal cell types (amnion,[21] testicular germ cells[22]), and neoplastic cells (K562 human leukemia cell line[23]) do not express Class I histocompatibility antigens. Incubation of the human amnion cells with gamma interferon caused both Class I HLA and $beta_2$ microglobulin expression.[21] Similarly, the K562 leukemia cell line, when incubated with interferon or sodium butyrate, developed significant HLA Class I expression.[23]

Rosa has suggested that gamma interferon plays an essential role in antigenic expression in amnion cells by its action at the DNA promotor level.[24] Similarly, Kawata has reported that HLA antigenic expression by fetal cytotrophoblasts is transcriptionally controlled.[25] Since stimulated T-lymphocytes are the source of gamma interferon, these lymphocytes may indirectly modulate donor antigenic expression. We have shown that selected grafts from early in the third trimester of gestation are not rejected. With the development of a blood supply to the graft from the host within 48–72 hours, for example, T-cells may come in contact with the graft, produce interferon, and stimulate the expression of Class I antigen, leading to full-scale allograft rejection. Such a scenario could partially explain the delayed fetal graft rejection found in our experiments but is not the full explanation, since grafts from later in gestation are so promptly rejected. Mechanisms active in graft rejection in the fetus might be applicable to treatment of certain types of neoplasia. A number of tumors lack surface antigen. Modulation of Class I antigenic expression, either with a naturally occurring substance (one of the interferons) or by a chemical (sodium butyrate), could allow the neoplastic cell line to be recognized as foreign and thus induce rejection.

In summary, fetal allograft acceptance could reflect the ontogeny of surface antigen expression in each fetal organ, which in turn could be modulated by growth factors or T-cell factors. These might influence promotors for mRNA expression or may directly act on the Class I surface antigen itself, either by effecting a configurational change or by preventing or delaying its glycosylation. Lau et al., for example, have demonstrated apparent alteration of the surface antigen of pancreatic islet cells after exposure to a short period of ultraviolet light.[26] The results of their treatment suggest a configurational change in antigen but a maintenance of islet function, since the cells, when transplanted into adult allogeneic recipient diabetic rats, caused a reversal of the host animals' hyperglycemia.

Detailed studies need to be carried out to interrelate Class I antigenic structure, function, recognition by the T-cell, and modulation by cellular growth factors. The methodologies of immunohistochemistry and molecular biology will help to further evaluate surface antigen development and factors that affect its expression. Modulation of the donor organ, both fetal and nonfetal, might allow a broadening of the pool of potential organs, an increase in the rate of successful transplantation, and diminished complications, since the overall immunologic response of the host may be less suppressed.

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Discussion

DR. JOHN A. MANNICK (Boston, Massachusetts): I would like to thank Dr. Foglia for presenting me with a copy of the manuscript, which I enjoyed reading. I must say that with the current legal climate in the United States, I am a little skeptical that fetal organ donation is likely to become a clinical reality very soon. This paper nevertheless addresses an important point in transplantation biology.

Earlier work in this field has clearly shown that some fetal tissues are easier to transplant than others, and there has been the general impression that the earlier the tissues are harvested in gestation, the better the chance they had for survival. This work very nicely shows that there is a marked difference in rejection response to liver *versus* kidney *versus* gonad, and that the earlier the organ is harvested in the gestational period, the longer the tissue survives.

My question for the authors is: Why did they choose to use an outbred set of rats? With the use of outbred rats, differences in survival can sometimes be altered by chance compatibilities of donors and recipients. I wonder if they would consider repeating their work using two inbred rat strains that differ in the major histocompatibility complex so that in each experiment there will be a similar transplant rejection response elicited by the foreign histocompatibility antigens that are present on the transplanted tissue. They also would have the monoclonal antibody tools available to dissect the very important question in this whole issue, and that is, what is the representation of the transplant antigens on the tissues that are transplanted? I think they should particularly look for the representation of the Class II antigens that trigger the transplant rejection response.

DR. CHARLES A. HUFNAGEL (Washington, D.C.): I rise to congratulate Dr. Foglia and his group for a very nice presentation of a complex problem.

It has been well demonstrated that different organs develop their immunological maturity at different times during gestation, but in general one can say that the period of immunity from the maturity of the immunosystem is basically the first half of gestation. The differentiation of organs is also very poor in many organs during that period. The fact that the kidney cells showed some maturity after transplantation is a very helpful contribution by the authors.

The basic issue, however, which Dr. Mannick expressed, is that it has been demonstrated that fetal tissue can be transplanted to an adult. The endocrine tissues, which secrete directly into the blood stream, require no organoid representation, and organs like the kidney have to be fully mature and have all the right connections to make urine. Its endocrine function is a different matter. I have five adult patients, two with Addison's disease and three with other endocrine deficiencies, all of whom have shown that physiologically they require no support for up to a year. Reports on some of those patients have been lost to follow-up.

The real problem is organ procurement. Meadowar demonstrated very well that a fetus is an available recipient for organs from the mother, which does not help anybody very much. On the other hand, what we need is a reverse Meadowar, to make animal donor chimera. That could be done by making a strong antibody to the organ and then injecting the fetus with the antibody. Can we really make this species step across that barrier? In amphibia subspecies, bridges have already been demonstrated, and cloning of amphibia and mice has been demonstrated with nucleus transplantation. This cannot yet be done after the gastrulation stage of development. This again is not a very practical matter except in animal species.

The real challenge still remains that the major source of donors for transplantation must ultimately be from an animal source probably by manipulation of the fetus or germ plasm. That will certainly be a fertile field when we start making that step.

DR. DAVID E. R. SUTHERLAND (Minneapolis, Minnesota): This paper is an interesting addition to an extensive literature on fetal allotransplantation. A group in Australia has consistently been able to engraft 12-day-old fetal mouse pancreases in diabetic mice and cure the diabetes if they do manipulations to prevent rejection, which includes tissue culture. However, fresh allografts invariably fail and do not cure the diabetes. Thus, at least 12-day-old fetal mouse pancreas retains its immunogenicity. They have also done work showing that there is expression of histocompatibility antigens in that stage of development, and I wonder if the authors have looked at their tissue for expression of histocompatibility antigens by the immunocytochemical techniques.

Also, as far as the human work is concerned, there have been about 100 or so fetal pancreas transplants performed in China in diabetic patients and about 50 in Russia that have been reported to the International Pancreas and Islet Transplant Registry, with some claims of function in the absence of immunosuppression. However, in Australia, of 20 or so clinical fetal pancreas transplants, there have been no cures of diabetes with or without immunosuppression.

DR. ELTON WATKINS, JR. (Burlington, Massachusetts): Twenty years ago, before I had a Human Studies Committee, I did vascularized fetal