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Fetal intestinal transplantation: use of immunosupressive agent in syngeneic hosts

Tansu SALMAN, Haluk GÜVENÇ, Hamdi KOÇER, Esma SÜRMEN, Nejat SAVCI, Türkan ERBENGİ, Alaaddin ÇELİK, Figen ERYÜREK, Tuncay ALTUĞ
Istanbul School of Medicine, Department of Pediatric Surgery, İstanbul.

Summary

Although fetal tissues are believed to be immunologically priviledged and would cause less graft versus host disease (GVHD), the use of immunosupressive agents is still not clear. The purpose of our study is to determine the effect of immunosupressive agents on fetal tissue transplantation among syngeneic animals. The first group consisted of twenty Wistar albino rats as recipients, which had a subcutaneus intestinal transplant, taken from a syngeneic donor at 15-17 days of gestation. Second group carried the same spesifications, only additionally received cy A 25 mg/kg subcutaneusly throughout the study. During the fourth week following transplantation, mononuclear invasion was observed at ultrastructural level in group one specimens, whereas specimens of the second group saved their constitution within normal limits. Results of our study investigating the spesific activity of gamma-glutamyl transferase, considering brush border activity of the transplant, showed that there was no significant difference between the two group's values (14.7 vs. 10.9 IU/gr. prot. p>0.05 respectively). The mitotic activity of the crypt cells of transplants, calculated by using vincristine arrest technique, were similar in both groups. As a conclusion, according to electron-microscopic studies alone, the use of immunosupressive agents may be necessary in fetal tissue transplantation, even if the animals used are syngeneic.

Key words: Fetal transplantation, fetal immunotolerance, intestinal transplantation.

Hiperalimentation is the only supportive cure for the short gut syndrome at present. This support is obligatory for the patient, since the time that will enable the remainnig intestinal segment to adopt the new physiologic conditions, by means of enlarged surface area, provided that villus hipertrophy occurs [21]. Hiperalimentation however, is expensive, difficult to manage by ambulatory means and has high morbidity. Intestinal transplantation is accepted as the alternative cure for the disease. Trials at clinical stage upto date has failed to succeed becauesa of technical difficulties as vessel anastomosis, or immunological problems as rejection, accompanied by graaft versus host disease (GVHD) [1,4,8,9,19]. Authors like Zinzar [22] have pointed out the advantages of fetal tissues, which are immunotolerable, will cause less GVHD and are potentilally able to neovascularize. Studies concerning fetal tissue transplantation between different genetic models have succeded so far under immunosupression [5,7,18]. In our former study we had been able to show that fetal intestinal segment of a rat transplanted into a sayngeneic host would establish blood suply, grow progessively and exhibit normal cytology [16,17]. The use of an immunosupressive agent in these studies is still not clear. The purpose of this study is to determine the effect of such an immunosupressive agent on fetal tissue transplantation among syngeneic animals, concerning ultrastructural level, functional capacity and mitotic activity.

Material and Methods

a. Transplantation: All transplantation experiments were done with syngeneic animals. The study consisted of two groups. In the first group twenty 30 days old Wistar albino rats (30-50 grams) were used as recipients. Fetii of pregnant Wistar albino rats were removed at 15-17 days of gestation. Their small intestine was removed en bloc, mesentery stripped off and the inside flushed with Ringer's lactate solution at 4 oc. This intes-

tinal tube (0.8 mm in diameter) was then placed heterotopically under the abdominal wall of the recipient. The second group also consisted of twenty other syngeneic hosts at the same weight, having undergone the same procedure, but additionally these animals received Cy A 25 mg/kg (Sandimum 50, Sandoz) everyday throughout the study. The intestinal transplants were irrigated in both gruops during second week.

b. Light microscopic and ultrastructural study: Tissues for microscopic examination were fixed in buffered glutaraldehyde-formaldehyde solution than processed using conventional technique for light microscope and electron microscope at second, third and fourth weeks.

c. Biochemical measurements (brush border activity): Spesific activity of the enzyme Gamma-glutamyl transpeptidase was examined using Rosalki's method [15]. The enzymatic activity was experessed as international units (micromoler p-nitroanilide, liberated per minute) per gram protein. Protein content of the homogenate was studied according to Lowry et al [13].

d. Mitotic activity: Vincristine induced metaphase technique was used [10,20]. Animals were given vincristine sulfate (Oncovin) 1 mg/kg body weight, intraperitoneally between 9 AM and 10 AM Animals were killed after 30 minutes. Tisse was fixed using conventional methods for light microscope. Mitotic activity was expressed in number of cells arrested at metaphase per 100 cells counted in a single area.

e. Radiologic study: In five rats abdominal aorta was cannulized twenty days after transplantation. 5-10 ml of micropague (100 %w/v, BaSO4 Pl 0838/0002) was infused via abdominal aorta. Following fixation of the transplanted segment in formaldehyde solution, the neovascularized zone was demonstrated radiologically by mammograph (25 volts and 50 mAs).

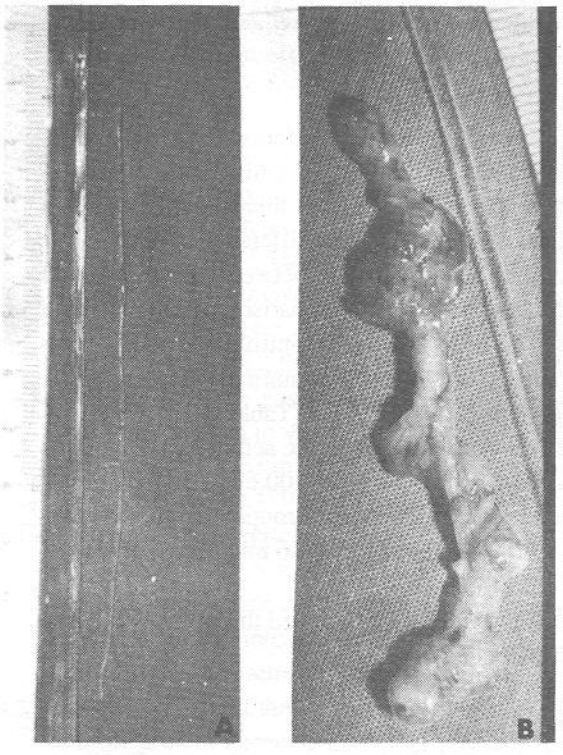

Fig. 1. Fetal intestine, A: before transplantation B: intestinal transplant after the second week.

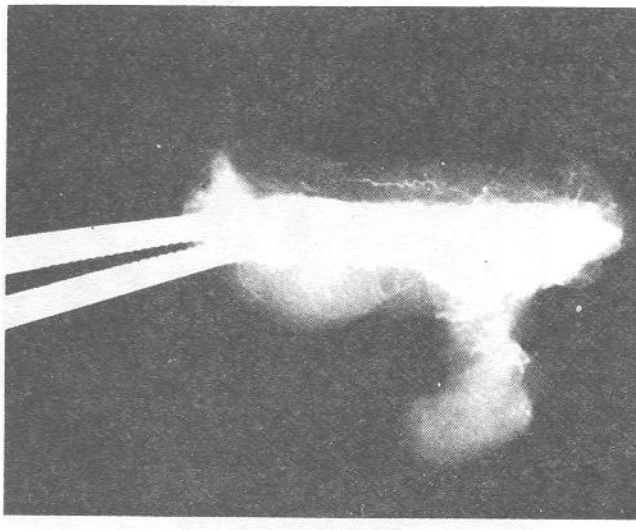

Fig. 2. Neovascularisation of the fetal intestine demonstrated after 20 days.

Results

a. In the first group, 16 out of 20 (80%) transplanted segment continued to grow and increase in diameter. (Fig. 1) In the second group, 18 out of 20 (90 %) segments survived. The remaining that failed to grow were accepted to result from techincal failure. In our former study we have observed that transplants distanted following study we have observed that transplants distented following second week of transplantation becaues of the accumulation of the segments secretory fonction, thus resulting in flattening of the vili and compromising intestinal segments blood supply [17]. We tried to avoid this by irrigating every segment during second week that followed transplantation. After irrigation, the content of the

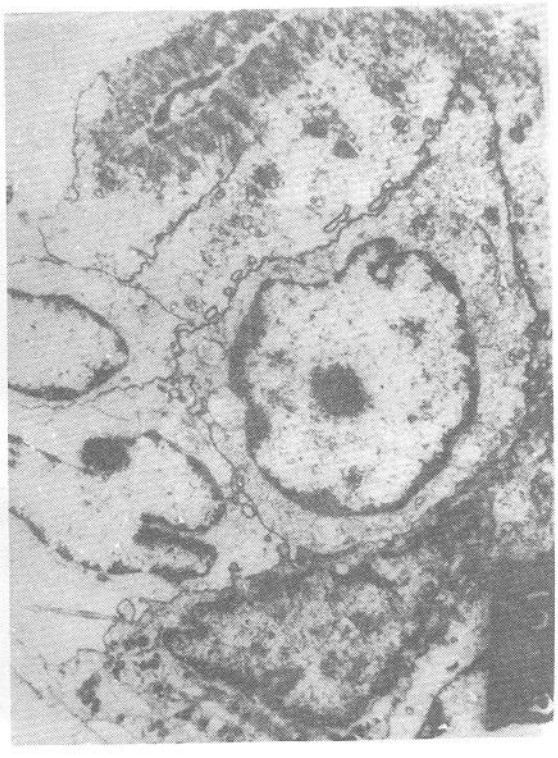

Fig. 3. Electron microscope, Mononuclear invasion seen during fourth week in group one animals. Cy A not used. (10100x)

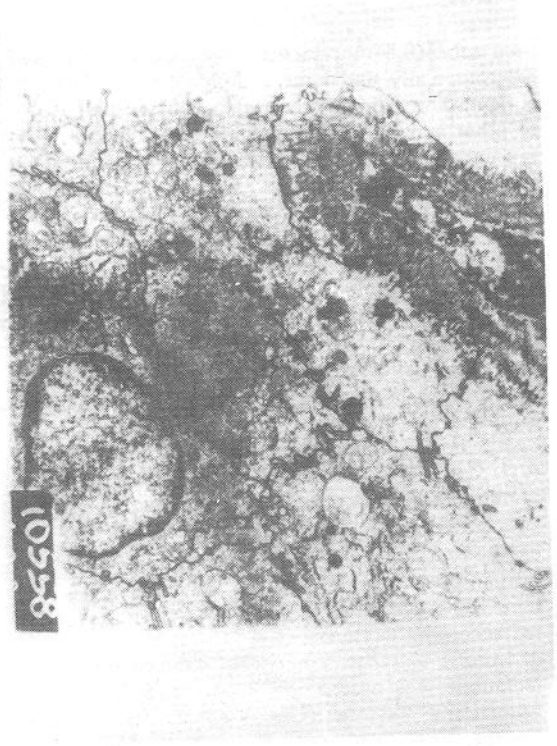

Fig. 4. Electron microscope, neogut columnar epithelial cells with basal nuclei and rough endoplasmic reticulum with mitochondria in the apical cytoplasm, in group two animals, during fourth week. These animals were given Cy A. (10100x)

segment had a mucoid character therefore avoiding overdistension.

b. Both groups revealed normal histology throughout the study, similar to normal ileum, having an intact mucosa composed to mixed epithelial cell types, submucosa and a bilayer muscular coat. No such evidence as mononuclear infiltration, flattening villi or disrupted mucosa with hipertrophied muscular coat, which are prominent signs showing rejection [12], was seen. At ultrastructural level a monouclear invasion was clearly seen during fourth week (Fig 3), a sign of rejection process seen only in group one animals. Electron microscope studies showed that this process started by the end to the third week. Sections examined from group two trasplanted segments under immunosupperssive treatment, showed neogut columnar epithelial cells with basal nuclei and rough endoplasmic reticulum with mitochondria in the apical cytoplasm. Dense microvilli with graunlosed endocrine cells (resembling cell type A) showed functioning intestinal segment, no mononuclear invasion was seen (Fig 4).

c. Gamma-glutamyl transferase is an enzyme responsible for trasport of aminoacids in the lumen. Data obtained from this study showed that there was no significant difference in the spesific activity of the enzyme between group one and group two. Where comparison of the results of the both groups with controls showed significantly lower levels than normal rat ileum either immunosupressed or not (Table I).

d. For both groups mitotic activity were counted to be 10-16 mitosis per 100 cyrpt cells. The mitotic index for control groups (host's own segment) were found to be 4-6 mitosis per 100 cyrpt cells (Table 2).

e. Radiologic study showed the well established neovascular zone. (Fig 2)

Discussion

The use of fetal tissues in transplantation has attracted ever incerasing interest since the firs pub-

Table 1. Specific activity of Gamma-glutamyl transferase

Group	(n)	GGI (IU/gr, prot)
1. Transplant	10	14.731±4.291
2. Transplant+Cy A	10	10.918±4.144 *
Wistar albino	5	32.950±10.699"
Wistar albino+Cy A	5	34.997±9.306 **

in comparison to group 1:* insignificant (p>0.05)
in comparison to group 1:" significant (p<0.01)
in comparison to group 1:** significant (p<0.01)

Table 2. Mitotic activity

Group	(n)	Mitotic index/100 cell
1. Transplant	5	13.2
2. Transplant+Cy A	5	12.4
Wistar albino	5	4.8
Wistar albino+Cy A	5	4.6

lication by Zinzar et al [22] in 1971. Authors like Foglia [6] pointed out the immunologic priviledge of fetal tissues and stated the uselessness of immunosupressive agents in syngeneic animals. It was also stated that factors like growth hormone have adjuant effect in host's immunotolerance [2,6,23].

In our previous study 69.09 % vitality was obtained, where only 63.15 % of these viable segments continued to grow, remainder losing lumenal integrity [16,17]. Using fetii with smaller gestational age we achieved a higher rate of success (80% for group one and 90% for group two). Zincar indicates that the vascular endothelium of adults has a rather passive tissue renewing very slowly, in contrast to vascular endothelium of fetus which develops much faster [23]. Montgomery and his friends [14] state that cellular maturation in intestinal system starts at seventeen days of gestation. Therefore we believed that younger fetii will have more potential to neovascularize and the immature intestine will adopt more easily to new host. Our aim in studying with younger fetii was to benefit the relative higher concentrations of growth hormone and other possible factors. By the end of the second week following transplantation, the intestinal tubes were visible and palpable from outside. We opened and irrigated the transplants; this procedure resulted in better survival of the transplant since the increasing intraluminal pressure led to flattening of the villi and later on disrupted histological integrity [16,17]. In these animals we created stomas to irrigate the segments from time to time.

Radiologic studies showed the vascularity of the transplanted intestine (Fig 2)
Histological studies showed that neovascularized segments were normal with no mononuclear infiltration, however, ultrastructural studies revealed mononuclear invasion starting by the end of the third week in nonimmunosupressed animals. Ultrastructural studies showed no trace of rejection throughout the study (Fig 3 and 4). It can be believed that as fetal tissue maturates the immunotolerability subsides, probably because of better differantiation producing stronger antigenic stimulance for the host's immune system. This is perhaps why fetal tissues fail to function unlike expected.

The purpose of intestinal transplantation is to provide a fonctioning intestinal segment for absorbtion of nutrients necessary. Therefore the functional capacity of the fetal segment is important. To investigate the brush border activity, we examined the specific acitvity of gammaglutamyl trasnferase, an enzme responsible for trasportation of amino acids [15]. Results of the study showed that levels were significantly lower, almost half the control groups, for both study groups (Table 1). Reviewing published studies concerning brush border enzymes we found that results were much simillar [3,11,18]. Some authors have stated that reason for this may be the lack of substrate situmaltion for the closed segment [18]. Others proposal concern lack of maturation of the fetal segment, or the disrupted lymphatics [14,18]. We belive that antigenic stimulance of the maturing fetal tissue, supersedes its immunotolerability thus resulting in poor functioning. The rejection process in syngenic host, may hamper the the trasplanted segments functional capacity; in our study, however there was no significant difference between the two grounps (Table 1). The reason why immunosupressed

trasnplants do not reach levels of specific acitivity when compared to nonimmunosupressed counterparts may be their immaturate state; maturation therefore may enhance the brush border activity.

Intestinal cells have a higher cellular regenaration rate when compared to others. Mitotic activity shows the capcity of DNA synthesis so mitotic activity of the transplant may provide us a better guideline for maturation when compared to histologic study alone. In both grups mitotic acitvity is similar and rate of mitosis seves a high level, as expected in fetal tissues (10-16 per 100 cells). The mitotic acitiviy of the hosts' own intestinal segments are higher (4-8/100 cells) when compared to adult levels (1-4 per 100 cells) which confirms that fetal tissues continue to grow with a high mitotic index well above newborn levels. Although the difference between the two groups was not significant (Table 2), We belive that mitotic index still may be a good parameter in folalwing the transplant's vital capacity. We are planning to conitnue this part of the study in larger animal groups.

As a conclusion, the ultrastructural study alone is evident enough to show the need for immunosupression in fetal intestinal transplantation between syngeneic models. We believe that such a transplanted segment, may serve as an accessory enteral segment, providing enough time for host's own remaining intestinal segment to adopt the new physiological state. Possible immunologic effects on functional capacity of the intestine and mitotic activity require further investigation.

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NOTICE !

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For correspondance:
Prof. Dr. Daver YEKER
Dept. of Pediatric Surgery
Cerrahpaşa Medical Faculty
34303 Cerrahpaşa
Istanbul-TURKEY