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Influence of Graft Encapsulation on Host Immune Activity. In *in vitro* Studies

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The aim of this study was to define the response of recipient immune system on free and encapsulated xenografts. Splenocytes and islets obtained from rats were encapsulated the Sun's method. Recipients were sensitized by i.p. injection free or encapsulated grafts. To evaluate host immune activity one-way Mixed Lymphocytes Cultures (MLC) Test were performed. Inactivated rat splenocytes or islets were used as stimulators and splenocytes obtained from naive and sensitized mice as responders. Increase of arousal splenocytes obtained from sensitized recipients were observed. Applied membrane did not prevent antigens penetration through capsular wall and stimulation of host immune system occurred.

K e y w o r d s: microencapsulation, immune activity, MLC test

1. Introduction

For prevention of allograft rejection a life-long immunosuppressive therapy must be applied. Encapsulation of insulin-producing cells in semipermeable membranes has the potential to provide an effective treatment for insulin-dependent diabetes with little or no immunosuppression of the host [1]. Improvements in alginate , a marine polysaccharide commonly used for cells encapsulation, have revived interested in this method. The method described by ZP Lim and AM Sun in 1980 [2] has been based upon the use of anionic acidic polysaccharide alginic acid, with gels on contact with a solution of calcium chloride. However, serious obstacles including a reliable

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cell source and a better understanding of immune acceptance issues remain to be addressed before a clinically applicable therapeutic procedure based on encapsulated islets becomes available. Intravascular and extravascular immunoisolation devices are studied by researchers for application in diabetes [3, 4].

Microcapsules have been the most intensively studied extravascular devices because of the spherical shape and small size that offers an optimal surface to volume ratio and an optimal diffusion capacity [5, 6].

The aim of this study was to evaluate whether xenotransplantation of rat splenocytes and islets encapsulated by alginate/poli-L-lisine/alginate membrane to mice would specifically stimulate the host immune system to verify the membrane performance in the system with encapsulated material capable to the specific stimulation.

2. Materials and Methods

Six- to eight-week-old male WAG rats were used as islet and splenocyte donors and 8- to 10-week-old, healthy Balb/C mice were used as recipients. The rat islets were isolated from the pancreases using the Lacy and Kostianowsky [7] method and purified with the Ficoll (1.083 g/mL; Sigma) density gradient. The rat splenocytes were obtained from spleen [8] and purified with the Ficoll (1.093 g/mL) gradient. The islets and splenocytes were cultured overnight in RPMI 1640 in a humidified atmosphere with 5% CO_2 and then encapsulated according to the three-step Sun's coating method with calcium alginate-poly-L-lysine-alginate using an electrostatic droplet generator [9]. Sodium alginate microbeads were collected by air extrusion into 1.1% $CaCl_2$. The islets encapsulated this way were washed several times with 0.1% CHES (Sigma) and suspended in 0.05% poly-L-lysine (Sigma), then washed with 0.9% saline, and suspended in 0.15% sodium alginate. After washing with saline they were put into 5 mM sodium citrate and finally washed with 0.9% saline and culture medium. The microcapsules were approximately 300 μm in diameter and of regular shape.

After the overnight culturing in RPMI 1640 at 37° C in atmosphere with 5% CO_2 *in vitro* viability of the free and encapsulated splenocytes or islets were assessed.

The recipients were immunized by intraperitoneal transplantations of: 10^6 free (F) or encapsulated (E) splenocytes (S); and 500 free or encapsulated islets (I). 10–14 days after grafting from immunized mice the splenocytes were obtained and the mixed splenocytes cultures tests were performed. The one-way mixed splenocyte culture (MLC) assay were performed by the standard method [10, 11]. Briefly, the responder cell suspension was adjusted to $5x10^5$ ml^{-1} of RPMI 1640 medium (Lublin). Stimulator cells were used at the following concentrations: $5x10^5$ ml^{-1} rat's splenocytes, 20 rat's islets were used. Stimulators were pretreated with mitomycin C (5 μg/mL, Sigma) by 45'[12]. The ratio of the proliferative response of the experimental to the healthy naive mice splenocytes was considered as an index for the responder immune activity These results were expressed as Stimulation Indices (SI) which

has been calculated as the ratio of counts per minute (CPM) in stimulated samples versus CPM in non-stimulated controls. Data are given as means ± SD. Statistical significance of differences was calculated with an unpaired Student's t test were level of significance was defined as $p < 0.05$ [13].

3. Results

The research material were divided in the following groups:

A. Splenocytes received from the non-sensitized mice stimulated in test by free splenocytes (C_1S) or free islets (C_2I);
B. Splenocytes isolated from the sensitized mice: free splenocytes (FS), APA encapsulated splenocytes (ES), free islets (FI), and APA encapsulated islets (EI).

Results are presented in Table 1. Maximal stimulation of the recipients splenocytes were obtained after stimulating with the free islets C_2I: SI = 24.5 ± 4.6 SD. SI after sensitization with the free rat splenocytes were significantly ($p < 0.05$) higher than in the control group FS: SI = 6.9 ± 3.0 SD, C_1S: SI = 4.2 ± 1.6 SD. Decrease of SI was observed after stimulation with both encapsulated splenocytes and islets ES: IS = 2.4 ± 1.7 SD; EI: SI = 2.7 ± 0.7 SD. Also after sensitization of the recipients with the free islets SI was lower than the control FI: SI = 7.1 ± 2.1 SD but significantly ($p < 0.05$) higher than after immunisation with the encapsulated islets.

Table 1. Results of the one-way modified MLC tests in the particular groups. Stimulation Indices

Groups	No of mice	Stimulator in MLC test	Specific stimulation index
C_1S = nonimmunized	7	free rat splenocytes	4.25 ± 2.6 SD
FS = immunized with free rat splenocytes	7	free rat splenocytes	6.9 ± 2.9 SD
HE = immunized with encapsulated rat splenocytes	7	encapsulated rat splenocytes	2.4 ± 1.7 SD
C_2I = nonimmunized	7	free rat islets	24.5 ± 4.6 SD
FI = immunized with free rat islets	7	free rat islets	7.1 ± 2.1 SD
EI = immunized with encapsulated rat islets	7	encapsulated rat islets	2.7 ± 0.7 SD

4. Discussion

Transplantation of encapsulated and free xenografts differently altered the magnitude of stimulation indices. In recipients stimulated with free splenocytes the immune

response in MLC test was increased. Immune responsiveness of the splenocytes taken from the recipients immunized with the encapsulated grafts was subnormal. This was an unexpected finding, since it was well documented, that encapsulated islet xenografts stimulate the host immune system. The reason of this is not clear. It may depend on peculiarity of grafted tissues which contain not only graft specific antigenic determinants, but also may produce cytokines [14] of different activity, and different degree of penetration throughout capsulary wall. It is possible, that in groups ES, EI penetration of inhibitory cytokines throughout the capsulary wall was faster than that of the graft antigenic determinants. It is also possible, that encapsulation reduced the relative strength of transplanted splenocytes and the density of their immune determinants and therefore the host responses were less intensive, than after transplantation of the free splenocytes. Such production was already observed in other circumstances. The influence of interaction between alginate and splenocytes can not be excluded [15].

The different values of SI's in the particular groups prove that protective action of encapsulation depends not on complete immunoisolation of the graft. As in the case of allotransplants rejection a penetration blockade of destructive immune elements throughout the capsular wall protects the graft from rejection.

5. Conclusion

The results of *in vivo* and *in vitro* studies give convincing proof that xenograft encapsulation did not prevent specific stimulation of the host immune system.
The results of the MLC test proved that encapsulated islets admittedly have stimulating activity but lesser than free islets and splenocytes.

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