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Kupffer cell blockade prevents rejection of human insulinoma cell xenograft in rats

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Abstract

Alloantigens are recognized by T-cells in the context of both class I and class II antigen, but class II antigens predominate in the recognition of xenoantigens. Since class II molecules bind peptides derived from exogenous proteins that have been phagocytized and digested into small fragments by antigen presenting cells, in the present studies the effect of gadolinium chloride ($GdCl_3$)-induced Kupffer cell blockade on the survival of discordant insulinoma cell xenografts was investigated. Insulinoma cells isolated by means of collagenase from human insulinoma and cultured were transplanted through the v. portae into the liver of streptozotocin-induced diabetic, male, CFY inbred rats. In the control, streptozotocin-treated rats, the decrease in blood glucose level was only transitory, in contrast with the $GdCl_3$-pretreated diabetic rats, which remained normoglycaemic during the 2-week observation period. Histologically, in the liver and lung of rats pre-treated with $GdCl_3$, large areas of extensively proliferating insulinoma cells were seen, whereas no insulinoma cells were seen in either the liver or the lung of diabetic-control rats, not-treated with $GdCl_3$. These studies suggest that the Kupffer cells play significant roles in the recognition of xenoantigens and the induction of xenograft rejection. © 1998 Published by Elsevier Science S.A.

Keywords: Kupffer cell; Phagocytosis blockade; Gadolinium chloride; Human insulinoma xenograft; Prevention of xenograft rejection

1. Introduction

Human pancreatic islet allotransplantation encounters many unresolved problems. One of these is the lack of a procedure for the isolation of adequate numbers of islets from cadaver donors [1]. Xenogeneic islet transplantation may solve this problem, but the immunologic consequences of xenotransplantation remain to be resolved.

One of the differences between allo- and xenotransplantation is the mode of antigen presentation. Class I molecules preferentially bind peptides generated from cytosolic proteins, whereas class II molecules bind peptides derived from exogenous proteins that have been phagocytized and digested into small fragments by antigen presenting cells. Alloantigens are recognized by T-cells in the context of both class I and class II antigen, but class II antigens predominate in the recognition of xenoantigens [2].

We reported [3] that rare earth metal salts, among them $GdCl_3$, depress the reticuloendothelial activity, selectively blocking the function of the hepatic Kupffer cells [4,5]. Since macrophages are needed for antigen presentation by class II molecules, it was of interest to study the effect of macrophage blockade on the survival of xenotransplanted insulinoma cells.

2. Materials and methods

Male CFY inbred rats (Biological Research Centre of the Hungarian Academy of Sciences, Szeged, Hungary), 200–250 g in weight, were housed with free access to pellet food and water at all times. Streptozotocin (Lot 112H0303, Sigma) was dissolved in 0.1 M citrate buffer and immediately injected iv in a dose of 50 mg kg^{-1} body weight. The plasma glucose level, the weight of the animal, and the food and water intake were monitored. Blood samples were collected from the tail veins of non-fasted rats. Plasma glucose levels were analysed by the glucose-oxidase method, using standard reagents from Reanal (Budapest, Hungary).

For pancreatic islet cell xenotransplantation, human insulinoma cells were used. The insulinoma cells were

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isolated from enucleated human benign insulinoma (identified histologically) by modified collagenase digestive method [6]. The cells were cultured in Eagles medium at 37°C in a humidified atmosphere of 5% CO_2 in air. Media were changed three times per week and a measured aliquot from each sample was taken for insulin radio-immunoassay (Izinta, Budapest, Hungary). During the 2 week cultivation the insulin production of the cells were constant ($235+23$ ng ml^{-1}). The cultured insulinoma cells were collected and suspended in Hank's solution and 2.9×10^7 cells were transplanted into the liver by injection through the v. portae.

To induce macrophage blockade [3–5], 1 mg 100 g^{-1} body weight $GdCl_3$ (Prolabo, France) was injected iv 24 h before cell-transplantation.

For light-microscopic studies, specimens of liver and lung were fixed in 4% paraformaldehyde–0.1 M sodium phosphate, pH 7.4, and embedded in paraffin, and sections were stained with haematoxylin and eosin. Sections were also stained with rabbit anti-human insulin, somatostatin and glucagon IgG (DACO, Copenhagen, Denmark), using the peroxidase–antiperoxidase staining method.

2.1. Data analysis.

Statistical analysis were performed by Wilcoxon Rank Sum Test. Statistical significance was accepted at a level $P<0.05$. Each point represents the mean and SEM of eight animals.

3. Results

The incidence of diabetes following streptozotocin injection was 100%. Three days following streptozotocin injection the mean plasma glucose levels exceeded 22.5 mmol l^{-1} (Fig. 1) and remained at the same levels until grafting.

One day following insulinoma cell xenotransplantation, the blood glucose level was decreased in both the control, streptozotocin-treated rats, and the streptozotocin-treated rats in which Kupffer cell blockade was induced by $GdCl_3$ administration. However, in the control diabetic rats, the decrease in blood glucose level was only transitory (1 day following insulinoma cell xenotransplantation), in contrast with the $GdCl_3$-pretreated diabetic rats, which remained normoglycaemic during the 2-week observation period (Fig. 1).

The food and water intake increased significantly following the induction of streptozotocin diabetes, which in the control diabetic rats was only temporarily influenced by insulinoma cell xenotransplantation. However, in rats pretreated with $GdCl_3$, following insulinoma cell xenotransplantation, the food and water intake gradually normalized following cessation of the hyperglycaemia.

Two weeks following insulinoma cell xenotransplantation, dyspnoea developed in some rats pretreated with $GdCl_3$. Since some of the $GdCl_3$-pretreated animals died, both the control diabetic rats not-treated with $GdCl_3$ and the $GdCl_3$-pretreated rats were sacrificed.

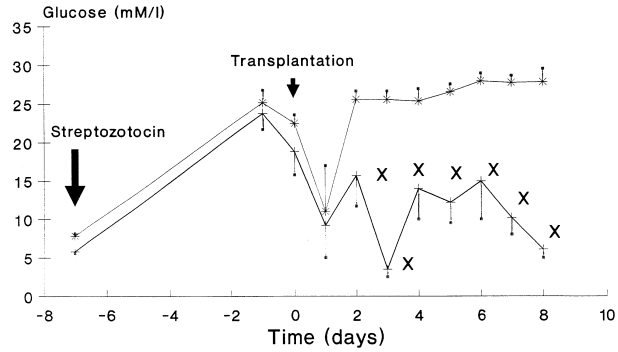

Fig. 1. Effect of insulinoma cell xenotransplantation on blood glucose levels. Streptozotocin (50 mg kg^{-1} body weight) was injected iv into both control and $GdCl_3$-pretreated rats on day 7. $GdCl_3$ (1 mg 100 g^{-1} body weight) was administered iv one day before insulinoma xenotransplantation, i.e. on day 1). The cells (2.9×10^7) were injected into the liver through the portal vein. Bar represents ±SEM. X=statistically different values ($P<0.05$).

Autopsy and histologic examinations confirmed the in vivo observations and explained the clinical signs of dyspnoea in the $GdCl_3$-pretreated rats. In the liver and lung of rats pretreated with $GdCl_3$, with minimal inflammatory cell infiltrations, large areas of extensively proliferating insulinoma cells were seen. The insulinoma nature of these cells was revealed by immunohistochemical studies, because they were positive for insulin and negative for glucagon and somatostatin. In contrast with the $GdCl_3$-pretreated rats, no insulinoma cells were seen in either the liver or the lung of control, diabetic rats not-treated with $GdCl_3$. In this group of rats, only inflammatory cellular infiltrations were present around the liver sinusoids.

4. Discussion

At the current time, the success of clinical transplantation has led to the demand for organs greatly exceeding the supply. The allotransplantation of pancreatic islets runs into similar problems. Xenogeneic islet transplantation may solve this problem, which stimulates research in this field. The rejection response to a xenograft, however, is usually vigorous and not adequately controlled by conventional immunosuppressive agents. The present studies show that Kupffer cell blockade induced by $GdCl_3$ prolongs the survival of a insulinoma cell xenograft implanted into the liver, which suggest that the Kupffer cells may play significant roles in the recognition or/and rejection of xenografts.

Originally, it had been published that $GdCl_3$ inactivates macrophages, as measured by reduced clearance of the test particles from the blood [3] and by decreased localization

of circulating particles to resident macrophages, such as Kupffer cells [4,5]. Recent evidence suggest that the number of macrophages is also lowered by intravenous $GdCl_3$-treatment. Gadolinium chloride injected intravenously reduces the macrophage-specific immunohistochemical staining of sections from rat liver and spleen [7]. The finding that the in vitro exposure of rat alveolar macrophages to $GdCl_3$ induces macrophage apoptosis [8], may explain our observation that $GdCl_3$-treatment allow insulinoma cells to proliferate not only in liver, but also in the lung.

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