Recovery of the immune system in diabetic mice after transplantation of isolated islets of Langerhans

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Summary. Adult CBA/HZgb mice islets harvested by collagenase digestion were injected intraperitoneally in 52 syngeneic diabetic recipients (CBA/HZgb→CBA/HZgb, 450–600 islets per mouse). Normal serum glucose levels, 24-h urine volume, insulin levels and body weight were completely restored to normal in all recipients during the next 2–5 months. Immunological function was assessed in control, diabetic and diabetic-transplanted mice by following their responses to sheep erythrocytes (expressed as the number plaque-forming cells in the spleen). In transplanted mice, the plaque-forming cell responses were as follows: 1 month after transplantation

– 43% of the plaque-forming cell counts in control (normal, non-diabetic) mice; 2 months after transplantation – 56% of the control value; and 94% of the control value after 5 months. Ten months after the transplantation, the plaque-forming cell counts were slightly above the control value (148%). It appears, therefore that transplanted islet tissue positively affects the immunological as well as the diabetic state of the recipients.

Key words: Islet transplantation, immune system, experimental diabetes, pancreatic islet isolation.

Bacterial and mycotic infections are common in diabetes mellitus [1]. Such infections are difficult to eradicate, and complicate management of the diabetic state. Numerous investigators have reported that the susceptibility of diabetic patients to infections should be ascribed to defects of polymorphonuclear leucocytes [2, 3]. Other investigators noted decreased numbers of T- and B-lymphocytes in peripheral blood and a depression of lymphocytic response to phytohaemagglutinin, suggesting impaired cell-mediated immunity in diabetic patients [4–6]. In a rat model, Mahmoud et al. [7] also found suppression of cell-mediated immunity. Pavelić et al. [8] described a decreased number of nuclear cells in thymus and spleen of alloxan diabetic mice. Brown et al. [9] and our group [10] described decreased antibody production to sheep red blood cells, a T-cell dependent antigen [9, 10]. Rejection of skin allografts was delayed in animals with experimental diabetes [8, 11]. According to our determination of E, E₃₇ or EAC rosettes in spleens of diabetic mice [12], defect of immunological function appears to be due not only to metabolic disturbances, but possibly also to altered proportions of the subpopulations of T and B cells. Daily injections of insulin into animals with experimental diabetes only partly restored the immunological function. This response was dependent on duration of the disease [13]. In this report

we compare reversal of the diabetic state in mice after transplantation of isolated adult islets from syngeneic donors with recovery of their immunological system.

Materials and methods

Animals and induction of experimental diabetes

Female CBA/HZgb mice, aged 2 and 4 months, were used as donors or recipients of isolated pancreatic tissue. Recipients were made diabetic by intravenous injection of alloxan (50 mg/kg body weight, Sigma, monohydrate, Lot. 50F-3823), prepared in phosphate-buffered saline (Hank's) solution. The drug was injected 14 days before planned transplantation. Mice received food and water ad libitum both before and after injection of the drug.

Blood glucose was determined by the method of Hyvarinen and Nikkilä, as modified by our laboratory [14]. Test-Tape strips (Glu-ketur-Test, Pliva, Zagreb) were used to check glycosuria. Only mice with glycosuria of >28 mmol/l were considered diabetic and were used in the experiment.

Isolation of islets of Langerhans

Islets of Langerhans were isolated from adult mice, aged 2 months, by the collagenase method of Lacy and Kostianovsky [16]. Following perfusion and distention of pancreatic tissue with 10 ml of cold (4°C) Hank's solution containing 0.68% neutral red, aprotinin (Trasylol, 500 units/ml, Sigma), the pancreas was excised, chopped into fine pieces with scissors, and digested in Hank's solution containing col-

count was 56% of that in control non-diabetic mice; at 5 months, it approached the control value (96%), and at 10 months it was even increased in islet recipients (48% above control value).

Discussion

Alloxan exerts a highly specific cytotoxic effect on the β cells of mouse pancreatic islets, and the resulting clinical condition closely resembles human insulin-dependent diabetes mellitus. In this model, using syngeneic mice and transplanting isolated islets, we demonstrated recovery of body weight, normalisation of glucose level in blood, disappearance of glycosuria, and rise of insulin concentration in serum.

The immune response to bacterial antigens is reduced in diabetic patients [2, 3, 5]. In addition to the correction of metabolic derangement, islet transplantation also resulted in recovery of immune competence. Treatment with insulin corrects metabolic status but the immune competence is only partly restored [14]. This may indicate that the immune system requires a full complement of islet hormones for its proper function, and that impairment reflects not only metabolic, but also a hormonal defect.

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