Virchows Arch [Pathol Anat] (1982) 396:61–71

Virchows Archiv A

Immunological Transmission of Glomerulosclerotic Changes in KK-Mice With Spontaneous Diabetes

1. Transplantation of Spleen Cells *

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Summary. To investigate whether glomerulosclerotic changes can be transmitted by spleen cells, two-month-old diabetic KK-mice received a spleen homogenate transplanted subcutaneously. The donors were two-year-old diabetic KK-mice. Control animals received physiological saline. Kidneys and pancreas were removed four or ten months after the transplantation. Apart from histological and partly immunohistological studies the kidneys (glomeruli) were also evaluated morphometrically on a blind basis. Blood sugar levels were determined together with serum insulin concentrations in some animals. Marked widening of the mesangium and an increase in mesangial cells was found in the transplanted animals four months after the transplantation when compared with the control animals, a finding that was confirmed by morphometric studies. All transplanted animals exhibited a lymphoplasmocytic periductulitis and some showed insulitis in the pancreas. Degranulation of β-cells was observed in some animals. Serum insulin was significantly reduced one month after the transplantation and blood sugar levels in the transplanted animals were continuously higher after three month than the values in the control animals.

The investigations show that transplantation of spleen cells induces progression of diabetic glomerulosclerotic renal alterations and also causes periductulitis and in isolated cases insulitis.

Key words: Diabetic glomerulosclerosis – Immune transfer – Insulitis – KK-mice

Mellors demonstrated in 1966 that the transplantation of spleen cells from old NZB/BL mice with membranous glomerulonephritis into young mice that

* Supported by the Deutsche Forschungsgemeinschaft (We 468/11-3)

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0340-1227/82/0396/0061/$02.20

were healthy before the transplantation induces glomerulonephritis. Buschard and Rygaard showed in 1978 that streptozotocin diabetes can be transmitted in mice by spleen T-lymphocytes. Kiesel et al. (1980) finally succeeded in transmitting experimental autoimmune insulitis in mice by transplantation of spleen cells.

We have taken up the train of thought of immunological transfer of diabetic changes resulting from these findings and have examined the question as to whether it is possible to induce acceleration or progression of glomerulosclerosis by transplantation of spleen cells from old diabetic mice with glomerulosclerotic renal changes into young KK-mice whose glomeruli do not show any glomerulosclerotic alterations.

Material and Methods

Experimental Animals

Six two-year-old diabetic KK-mice which exhibited typical diabetic renal changes were used as donors. The recipients were two-month-old diabetic KK-mice. All animals were inbred mice which had been bred by continuous mating of closely related stock. Control animals were likewise two-month-old KK-mice which received physiological saline by injection.

Transplantation

The spleen was obtained from the donor animals under aseptic conditions and triturated mechanically in sterile, buffered physiological saline at 4° C (approx. 1 mm^3). Every recipient animal received approximately 1/8 of a spleen through a sterile, wide-lumen needle into the ventrolateral abdominothoracic region injected subcutaneously.

Experimental Groups

A total of 28 two-month-old KK-mice were used for the experiments. Fourteen animals received a splenic transplantation. Seven animals were killed 4 months (hence aged 6 months) and 6 animals 10 months (aged 12 months) after the transplantation. One animal died during the study period. The other 14 animals served as age-matched controls which were likewise killed 4 or 10 months after the injection.

Laboratory Studies

Fasting blood sugar levels were measured in the animals at monthly intervals over a period of up to seven months. Measurements were carried out with the glucose UV test using the hexokinase method (Boehringer Mannheim). Blood was obtained from the tail vein. Furthermore, serum insulin concentrations were determined in each of five animals one month after the transplantation or the injection of physiological saline to detect any changes as soon as possible. These measurements were carried out by radioimmunoassay.

Histological Technique

Kidneys, spleen, muscle tissue (psoas), eyes, liver and pancreas were removed immediately after killing. The kidneys, spleen, liver, muscle tissue and eyes were fixed in 4% buffered formalin solution and the pancreas in Bouin. Parts of the kidneys were fixed again in 2% buffered osmic acid for 2 h and embedded in plexiglass. The other organs were embedded in paraffin. The PAS reaction was carried out on the paraffin sections, the sections of pancreas were stained using the HE and the aldehyde-fuchsin-trichrome technique, whereby the latter served to represent β-cell granulation. Sections of the material embedded in plexiglass 0.5–1 μ in thickness were prepared and then silvered using the method of Movat. HE staining was done on all other organs.

Immunohistology

Parts of the kidney were deep-frozen immediately following removal and sections 3–4 μ in thickness were prepared in a cryostat at −20° C which were subsequently studied immunohistologically using the direct method. FITC-labelled anti-mouse IgG from the rabbit (Cappel) were used as antisera (antibody protein 3.0 mg/ml, total protein 16.0 mg/ml, F/P ratio: 2.8 mg/g) and anti-mouse β_1C from the goat (Cappel) (total protein 14.8 mg/ml, F/P ratio 2.1 mg/g). The antibody specificity was checked by immunoprecipitation and the sera were employed in buffered solution at pH 7.2 at a dilution of 1:5. Apart from the albumin control, control sections were treated with unlabelled sera before studies were performed. The studies were carried out suing a Zeiss fluorescence microscope.

Morphometry

The morphometric evaluation was carried out on a blind basis on the semithin sections silvered according to Movat (Wehner 1981). Allocation of the results to the various experimental groups was not carried out until completion of the mathemtical analysis. We studied 20 different glomeruli per animal. The following variables were determined:

1. Mean glomerular and mesangial area, using the point counting technique and a Reichert Visopan, with a regular distance between points of 6 μ.

2. Total number of glomerular cells and their differentiation by direct counting in the microscopic picture on the ground-glass screen of a Reichert Visopan (Obj. 63 or oil immersion).

3. From the values we calculated the percentage mesangial fraction of the glomerular area, the percentage fraction of the total cell count and the glomerular and mesangial cell density (viz. total glomerular cell count per 1,000 μ^2 glomerular surface and mesangial cells per 100 μ^2 mesangial surface).

Statistics

The statistical analysis of the main results was carried out using Student's t-test. The limit for the error probability was $2\,p < 0.05$ (5%).

Results

1. Light Microscopy

The glomeruli of the transplanted animals showed marked thickening of the mesangium and an increase in mesangial cells four months after transplantation which appeared to be far more pronounced than in the control animals of the same age (Fig. 1). We also demonstrated peri-membranous changes of the basement membrane in some transplanted animals. The animals still exhibited marked glomerular changes 10 months after transplantation with aneurysmal widening of the capillaries and some nodular structures (Fig. 2).

The pancreatic islets of some of the transplanted animals showed marked degranulation of β-cells. All transplanted animals showed a periductulitis (Fig. 3). The inflammatory infiltrates consisted of lymphocytes and plasma cells in periductular tissue. Although the islets in mice mostly lie on or adjacent to ducts so that differentiation of periductulitis from pure insulitis is difficult, with a few exceptions the islets were found to be free from inflammatory infiltrates (Fig. 4) (Table 1). A malignant lymphoma developed in one animal with destruction of the pancreas and extensive infiltrates in the spleen. A periductulitis in the region of the islets was likewise present in four of six mice which were

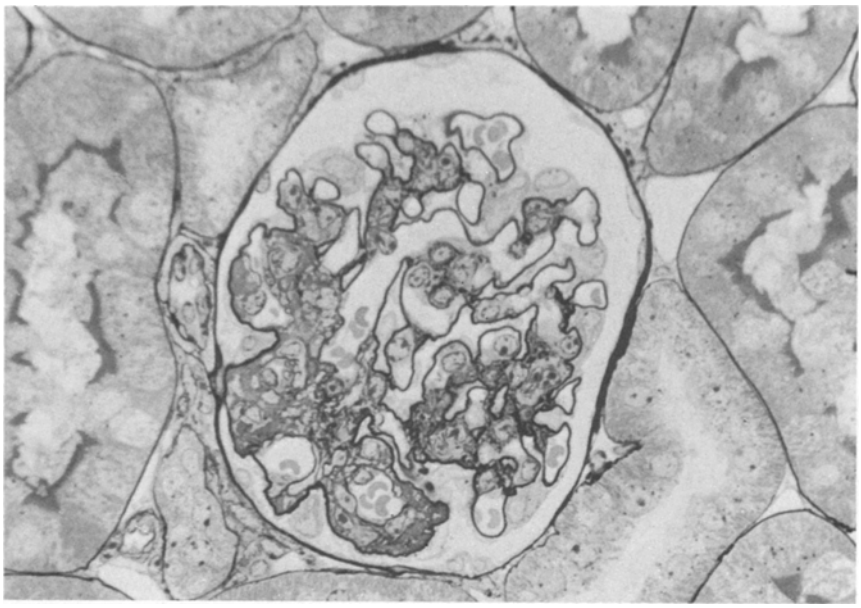

Fig. 1. Glomerulus of a KK-mouse 4 months after spleen cell transplantation with marked proliferation of mesangial cells and widening of mesangium. (Argentation according to Movat, 648:1)

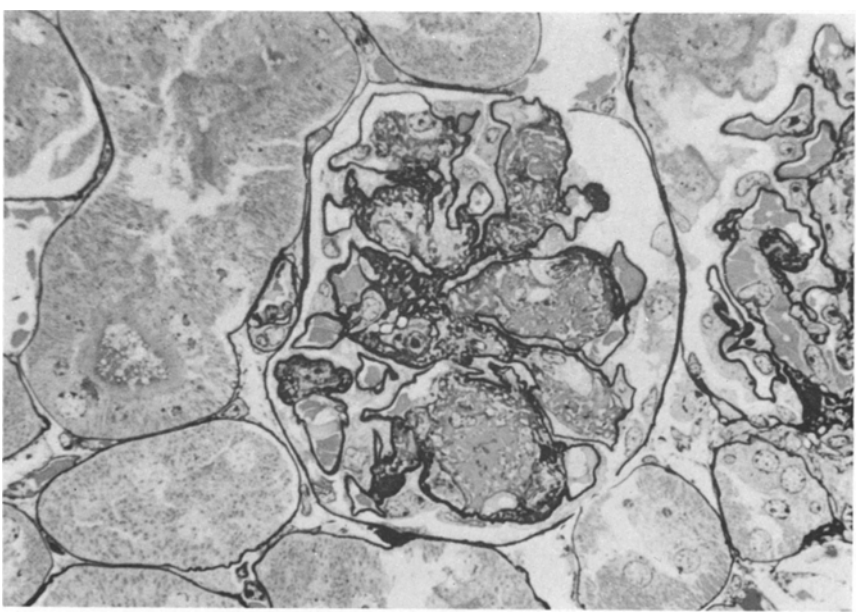

Fig. 2. Glomerulus of a KK-mouse 10 months after spleen cell transplantation with marked nodular alterations. (Argentation according to Movat, 648:1)

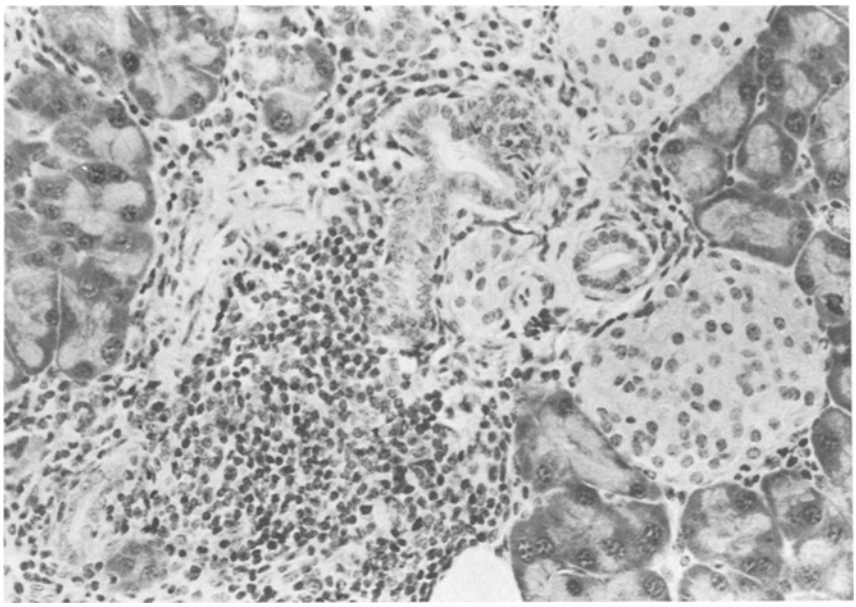

Fig. 3. Islets of Langerhans of a KK-mouse 4 months after spleen cell transplantation. Lymphoplasmocytic infiltrates in periductular connective tissue (periductulitis) (HE, 237,6:1)

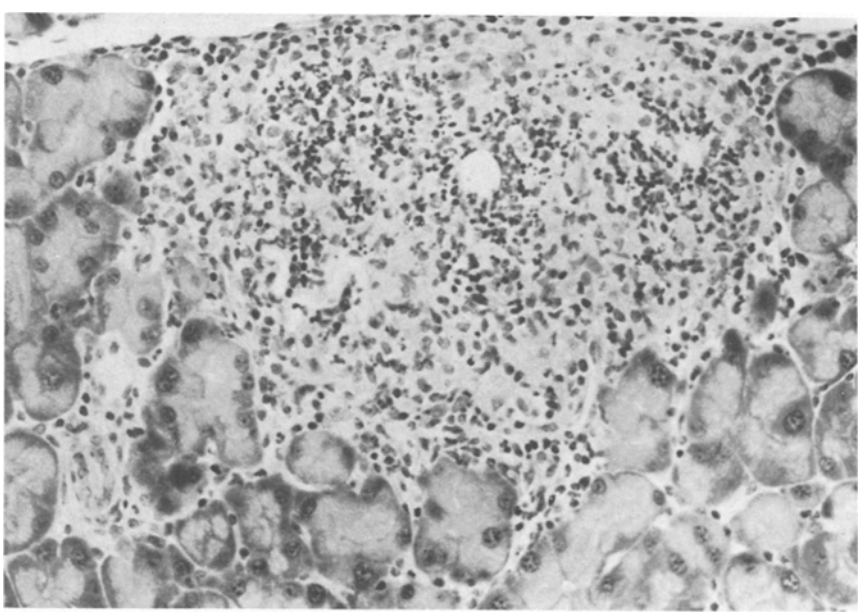

Fig. 4. Islets of Langerhans of a KK-mouse 4 months after spleen cell transplantation with marked inflammatory infiltrates (insulitis) (HE, 270:1)

Table 1. Light microscopic changes of the kidneys, pancreas, liver and spleen in the various experimental groups

		KK Control (4 month) $n=7$	KK Transplantation (4 month) $n=7$	KK Control (10 month) $n=7$	KK Transplantation (10 month) $n=6$
Kidney	0	1			
Glomerulosclerosis	+	6	5	2	4
	++		2	4	1
	+++				1
Pancreas					
Periductulitis		0	6	0	4
Insulitis		0	1	0	0
Degranulation		0	5	0	2
Lipomatosis		1	0	0	0
Malignant lymphoma		0	1[a]	0	0
Liver					
Kupffer cell activation		1	2	1	1
Hyperaemia		1	0	0	0
Malignant lymphoma		0	1	0	0
Fatty infiltration		0	0	3	1
Haemangioma		0	0	1	0
Spleen					
Enlarged follicles		0	3	0	2
Increase in giant cells		0	1	2	0
Malignant lymphoma		0	1	0	0
Focalized round-cell infiltrates		0	0	0	1

[a] In one case with malignant lymphoma a periductulitis cannot be clearly differentiated owing to the lymphoma infiltrates

killed ten months after transplantation. We found only nonspecific changes such as Kupffer cell activation, fatty infiltration, hyperaemia and in one case a haemangioma in the liver of both the transplanted animals and the control animals. Significant infiltrates similar to those in the pancreas were not present. Lymphoma was found in the liver of the animal with the malignant lymphoma (Table 1). The spleen of some of the animals of the transplantation group showed enlarged follicles and some animals also exhibited an increase in giant cells (Table 1). The other organs – psoas muscle and eyes – were normal.

One of the 2 animals of the 10-month transplantation group without periductulitis showed mild and the other moderately severe glomerulosclerosis.

2. *Immunohistology*

Four months after the transplantation immunohistological studies showed the typical pattern of fine-granular depositions of IgG and β_1C along the glomerular capillary wall in the mice which exhibited light microscopic perimembranous

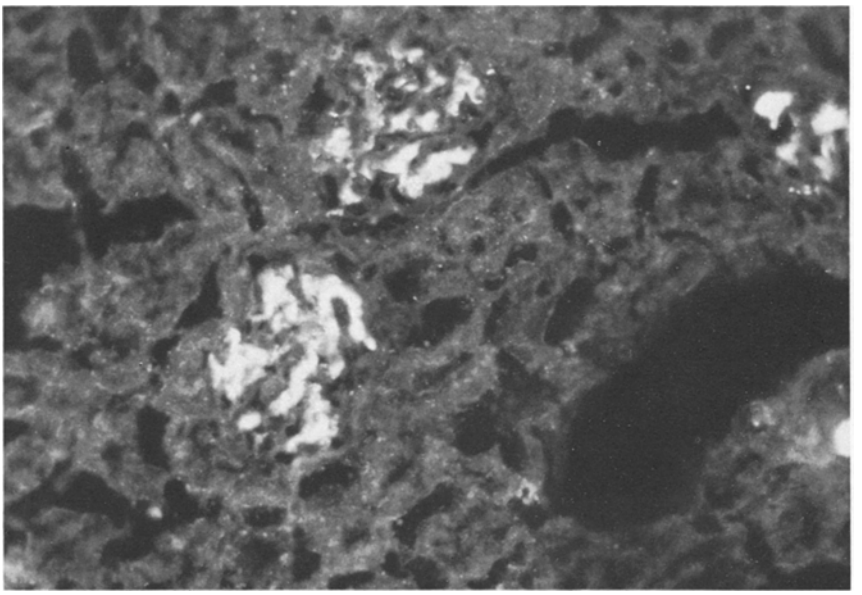

Fig. 5. Deposition of IgG predominantly in mesangium in a KK-mouse 10 months after spleen cell transplantation

changes. Marked IgG depositions and less marked β_1C depositions in granular clumpy form were present in the mesangium in the animals which were studied 10 months after the transplantation (Fig. 5). The immunohistological findings in the control animals were mostly less intense and particularly the fine-granular depositions along the capillary wall were not demonstrable in these animals.

3.Morphometry

Total Glomerular Cells. The total glomerular cells in the transplanted animals four months after the transplantation with a mean count of 46.6 cells were significantly increased as compared with a mean count of 32.8 cells in the control animals. After 10 months the cell counts were more or less the same again, namely 37.5 in the transplanted animals and 37.8 in the control animals (Tables 2 and 3).

Endothelial Cells. The mean absolute endothelial cell contents was significantly increased in the transplanted animals after four months, namely 18.3 as compared with 15 in the controls. Here too normalization occurred after 10 months. The endothelial cell content of the transplanted animals was 16.7 (44.4%) and in the controls 17.1 (44.8%) (Tables 2 and 3).

Mesangial Cells. The mesangial cells showed similar behaviour. After 4 months the mesangial cell count in the transplanted animals of 14.7 (35.2%) was significantly higher than the control value of 10.2 (31.4%). Here too normalization occurred after 10 months so that the value in the transplanted animals was

Table 2. Morphometric glomerular parameters ($\bar{x} \pm SD$) in KK-mice ($n=7$) 4 months after transplantation of splenic cells and in age-matched controls ($n=7$)

Glomerular parameters	KK Control	KK Transplantation	$p<0.05$
Total glomerular cell count	32.8±3.5	46.6±3.4	+
Endothelial cells	15.0±3.5	18.3±2.0	+
	(45.2±4.1)[a]	(44.8±4.6)	
Mesangial cells	10.2±1.1	14.7±3.2	+
	(31.4±3.1)	(35.2±5.3)	
Epithelial cells	7.4±1.2	7.7±0.1	+
	(23.2±2.7)	(19.7±1.9)	
Glomerular area (μ^2)	4,397±656	5,262±463	+
Mesangial area (μ^2)	507±75	692±160	+
Mesangium (%)	11.7±1.7	13.1±2.5	+
Glomerular cell density (total cells/1000 μ^2)	7.7±1.5	7.8±0.4	
Mesangial cell density (mesangial cells/100 μ^2)	2.0±0.3	2.1±0.3	

[a] ( )=percentages

Tabelle 3. Morphometric glomerular parameters ($\bar{x} \pm SD$) in KK-mice ($n=6$) 10 months after transplantation of splenic cells and in age-matched controls ($n=7$)

Glomerular parameters	KK Control	KK Transplantation	$p<0.05$
Total glomerular cell count	37.8±3.5	37.5±3.2	
Endothelial cells	17.1±2.8	16.7±2.0	
	(44.8±4.8)[a]	(44.4±2.8)	
Mesangial cells	13.8±1.9	13.0±1.6	
	(36.3±3.4)	(34.2±2.8)	
Epithelial cells	6.8±0.8	7.7±0.7	
	(18.8±3.2)	(21.4±1.9)	+
Glomerular area (μ^2)	5,759±463	5,747±367	
Mesangial area (μ^2)	750±78	697±10.4	+
Mesangium (%)	13.4±1.2	12.2±1.4	
Glomerular cell density (total cells/1000 μ^2)	6.7±0.4	6.5±0.7	
Mesangial cell density (mesangial cells/100 μ^2)	1.8±0.2	1.8±0.3	

[a] ( )=percentages

13 (34.2%) mesangial cells in a mean glomerular section and 13.8 (36.3%) in the control animals.

Epithelial Cells. The epithelial cells were significantly reduced in the transplanted animals after four months, namely 19.7% as compared with 23.2% in the controls, but after 10 months they were significantly increased, namely 21.4% as compared with 18.8% in the control animals.

Table 4. Mean blood sugar levels (mg%) in transplanted KK-mice and controls ($\bar{x} \pm s_x$)

Time (months)	KK Control	KK Trans-plantation	$p < 0.05$
1	149 ± 5.9 ($n=10$)	190 ± 6.6 ($n=12$)	+
2	174 ± 9.0 ($n=9$)	133 ± 8.0 ($n=12$)	+
3	152 ± 10.6 ($n=9$)	150 ± 6.8 ($n=11$)	
4	131 ± 6.5 ($n=4$)	146 ± 7.8 ($n=7$)	
5	138 ± 4.5 ($n=4$)	157 ± 14.4 ($n=4$)	+
6	118 ± 8.0 ($n=4$)	156 ± 14.0 ($n=4$)	+
7	126 ± 22.5 ($n=4$)	152 ± 17.3 ($n=4$)	+

Glomerular Area. Differences were observed after four months. The control animals had a mean glomerular surface of 4,397 μ^2; in contrast those of the transplanted animals were significantly greater: 5,262 μ^2. After 10 months the renal corpuscles in both groups were of practically the same size, namely 5,747 μ^2 in the transplated animals and 5,759 μ^2 in the controls (Tables 2 and 3).

Mesangial Area. There was a significant increase in the mean mesangial area with 692 μ^2 in the transplanted animals after four months compared with 507 μ^2 in the control animals. These values increased significantly in the control group during the further course of the study to 750 μ^2, whereas they remained practically unchanged in the transplanted animals with 697 μ^2 (Tables 2 and 3).

Percentage Mesangial Content. This response of the mesangium during the course of the study is also apparent from the percentage mesangial content which after four months accounted for 13.1% in the transplanted animals compared with 11.7% in the controls. After 10 months the situation was reversed: The percentage mesangial content in the transplanted mice was 12.2% compared with 13.4% in the control animals (Tables 2 and 3).

Glomerular and Mesangial Cell Density. These variables did not show any significant differences between the 2 groups and the 2 study periods (Tables 2 and 3).

4. Serum Insulin

One month after transplantation of the spleen the mean serum insulin concentration in five transplanted mice was 35.5 ± 4.1 μU/ml and in five controls it was 43.2 ± 3.8 μU/ml.

5. Blood Sugar

Initially there was an increase in blood sugar both in the transplanted mice and the control mice from 174 to 190 mg%. After 3 months the values were of about the same magnitude (150mg%). During the further course of the study the blood sugar level in the transplanted animals of more than 150 mg% was significantly higher than that in the controls, namely not more than 138 mg% (Table 4).

Discussion

It is evident from the findings that by syngenic transplantation of old spleen cells from diabetic KK-mice into young diabetic KK-mice progression or acceleration of diabetic glomerulosclerotic renal changes is induced. Later this alteration in the course of the disease reverts to normal the disease. At the same time we found a periductulitis but rarely an insulitis in the pancreas of the animals which had received a spleen cell transplantation.

The question of the specificity of the changes appears to us to be of importance, viz. whether a possibly nonspecific effect of transplantation is present. It is evident from a further experimental series in which we transplanted spleen cells from diabetic KK-mice into nondiabetic mice of another strain (NMRI) that these animals empirically exhibit a marked renal alteration in the sense of a serum nephritis. It was of interest – and this supports the specificity of our findings in the case of syngenic transplantation – that the animals exhibited neither diabetes mellitus nor changes in the islets. Since there is no such thing as nondiabetic KK-mice it is not possible to investigate any nonspecific effect of the transplantation in a nondiabetic inbred variant. Likewise studies on other organs (liver, musculature, spleen, eyes) did not furnish any evidence for infiltrates or other reactions so that we would like to assume that the changes described are the result of a specific effect of the transplantation on the renal and pancreatic alterations.

It is not possible from our studies to decide what cell population is responsible for this effect. Kolb et al. (1980, 1981) who transmitted streptozotocin-induced insulitis by spleen transplantation in B 6 and C 57 BL/6 J-mice postulate that spleen lymphocytes which induce a graft-versus host reaction are responsible. Kiesel et al. (1980) found an insulitis in 75% of their recipient animals but they did not demonstrate any hyperglycaemia. However in our investigations the animals that had received spleen cell transplants subsequently showed marked hyperglycaemia when compared with the control animals. Possibly this difference can be explained by the time factor and the species. The hyperglycaemia in our animals is undoubtedly a result of the severe islet damage (insulitis, periductulitis and degranulation of β-cells). Buschard and Rygaard (1978) also succeeded in transferring streptozotocin-induced diabetes mellitus in mice by splenic T-lymphocytes. They assumed that T-suppressor lymphocytes exert a specific effect. Kiesel et al. (1981) emphasize that the transmission of experimental immune insulitis is dependent on the strain of mice and that this is only successful in syngenic strains. Hence the identity of the main histocompatibility

locus is of essential importance for the cytotoxic activity of the transferred cells. On the other hand Flohr et al. (1981) have postulated that under certain circumstances the cellular immune response to islet cells can occur as a result of a general immune abnormality.

Morphological changes in the kidneys have not been described by these various authors. Based on our immunhistological findings we would like to interpret these changes as an antigen-antibody reaction. However, it is obvious that with transplantation, other changes can also occur within the scope of a cellmediated immune response (insulitis). Further investigations with transplantation of specially isolated cells, with transplantation of younger splenic cells and in athymic mice are necessary to explain the described phenomena in more detail.

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Accepted February 9, 1982