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Pancreatic cellular infiltrates in autoimmune-prone New Zealand Black mice

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Summary. Healthy New Zealand Black (NZB) mice of both sexes (age 19–31 weeks) were studied to determine the magnitude of pancreatic β cell injury related to mononuclear cell infiltration of islets. This investigation was undertaken following the description of spontaneous cellular immune reactions against islets in four strains of autoimmune-prone mice, including NZB mice [1]. Studies included complete autopsies with histological examination, determination of the pancreatic content of immunoreactive insulin, and the measurement of the plasma glucose concentration. Mononuclear cell infiltrates were identified in the lung, liver, kidney, salivary gland, mesentery, and pancreas. In the latter site, the infiltrates were situated in fibrous septae about ducts, ductules, and venules rather than islets. Only islets contiguous to infiltrates were involved, and then but focally. Insulitis, as manifest by the envelopment and permeation of islets by mononuclear cells, was not observed. In none was there a significant reduction of β cells or pancreatic insulin content, neither was hyperglycaemia manifest. This study reveals that, although NZB mice are subject to autoimmune phenomena and widespread mononuclear cell infiltrates, β cell injury and insulitis are not consistent features of this strain.

Key words: New Zealand Black mice, diabetes mellitus, autoimmunity, pancreatic cellular infiltrates.

The New Zealand Black (NZB) mouse has served as a model for a variety of autoimmune conditions. Originally derived from agouti mice following inbreeding for coat colour [2], NZB mice develop spontaneously (as a function of ageing) severe autoimmune haemolytic anaemia [3], mild immune complex glomerulonephritis [4], and various expressions of β cell lymphoproliferation, including lymphoma [5]. The intense study of this strain, as well as the Fl hybrid derived from the black (NZB) and white (NZW) strain, the animal model for systemic lupus erythematosus [6], has generated a wealth of information related to the ontogeny, regulation, and ageing of the immune system [7]. Although prone to age-related illnesses, the NZB mouse has not been described to develop diabetes mellitus, even though a related strain, the New Zealand Obese mouse, serves as a model of autoimmune-mediated, adult-type insulin-resistant diabetes [8].

A recent report described spontaneous cellular immune reactions against pancreatic islets with β cell destruction in NZB, (NZB × NZW) Fl hybrids, MRL, and BXSB mice [1]. The latter three strains manifest many autoimmune phenomena which include polyclonal β cell lymphoproliferation, the formation of autoantibodies and circulating immune complexes, abnormalities of Ig and complement, and a severe immune complex glomerulonephritis [9]. This report was provocative since spontaneous immune reactions against islets and β cells are uncommon events in mice, one notable exception represented by the non-obese diabetic mouse [10]. Intrigued by this finding, we undertook a study to assess the magnitude of pancreatic β cell injury in autoimmune-prone mice. Since the index report detailed islet lymphocytic infiltrates in 80% of NZB mice, 50% of (NZB × NZW) Fl hybrids and MRL mice, and less than 20% of BXSB mice, the NZB strain was chosen as our experimental model. Moreover, since adult mice had been reported to show a more intense form of insulitis than younger animals [1], we elected to study adult animals.

Materials and methods

Animals

Mice of both sexes of the NZB/BlNJ strain were utilized at 19–31 weeks of age. Controls were represented by male and female BALB/cJ mice, aged 15–27 weeks, and male and female CBA mice,

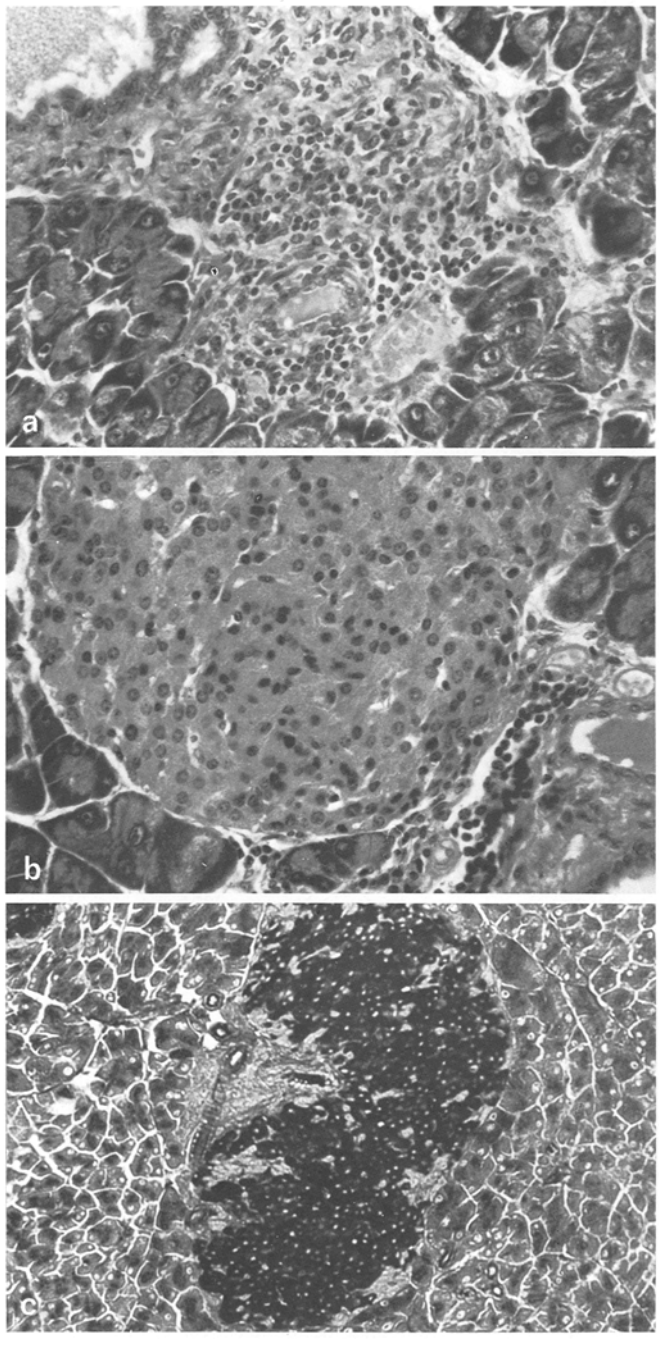

Fig. a–c. Photomicrographs of: **a** focus of intense pancreatic periductular and perivascular mononuclear cell infiltration (haematoxylin-phloxine-saffron, ×162); **b** pancreas demonstrating normal islet with minute focus of mononuclear cell infiltration at vascular pole (haematoxylin-phloxine-saffron, ×162); **c** islet with cellular infiltrate at vascular pole stained with aldehyde fuchsin technique illustrating well-granulated β cells (Gomori aldehyde fuchsin stain, ×81). This pattern of islet staining respresented a consistent finding

aged 28 weeks. The NZB/BlNJ and BALB/cJ mice were purchased from the Jackson Laboratories, Bar Harbor, Maine, USA. The CBA mice were donated from the colony of Professor W. S. Lapp, Department of Physiology, McGill University, Montreal. All animals received standard laboratory chow and water ad libitum and appeared healthy.

Study design

At intervals, animals (15–31 weeks of age) were killed with ether and tissue and plasma were obtained for analysis. All studies were performed on coded specimens.

Histology

Tissue from complete autopsies was fixed in 10% buffered formalin, except the pancreas which was fixed in Bouin's solution. Paraffin-embedded sections were stained by haematoxylin-phloxine-saffron and examined by light microscopy. Pancreatic sections were stained, as well, by the Gomori aldehyde fuchsin technique for β cells [11]. Approximately 90% of the pancreas was submitted for histological study; the remaining 10% (tail of pancreas adjacent to the spleen) was employed for insulin assay (vide infra). A minimum of ten step-sections was examined from each block of pancreatic tissue.

Tissue extraction and hormone assays

A portion of pancreas (approximately 10%) was excised, blotted, weighed, and placed in acid-alcohol solution. The piece was homogenized and left overnight at 3 °C. The homogenate was centrifuged at 4000 rev/min for 30 min and the supernatant was stored at −20 °C until assayed. Blood was collected in heparinized tubes from the orbital sinus. Plasma was separated and the samples were stored at −20 °C. Pancreatic immunoreactive insulin (IRI) concentration was determined as described previously [12]. Plasma glucose was measured by an automated glucose oxidase method (Beckman glucose analyzer 2; Beckman Instruments, Fullerton, California, USA).

Statistical analyses

The values of pancreatic IRI and plasma glucose from NZB mice were compared with those from control mice. Similarly, values from these two determinations were compared among NZB animals with and without pancreatic mononuclear cell infiltration. The data were analyzed by Student's t-test. A p value of > 0.05 was considered to be significant.

Results

Histology

Sections from all 16 control mice disclosed normal histology in all tissues. No cellular infiltrates were recognized in the pancreas; islets maintained a normal complement of β cells as judged from aldehyde fuchsin-stained sections.

Sections from 21 NZB mice disclosed mononuclear cell infiltrates in the lung (13 animals), liver (four animals), kidney (four animals), and salivary gland (three animals). The infiltrates were disposed about bronchi, bronchioles, ducts, ductules, and/or venules. Megakaryocytic hyperplasia was manifest in the spleen; follicular hyperplasia was noted in lymph nodes; no consistent alterations were recognized in the thymus. In several animals, nodular lymphoid aggregates were noted in mesenteric fat adjacent to the pancreas. In eight animals, discrete mononuclear cell infiltrates were distributed in fibrous septae about pancreatic ducts, ductules, veins, and venules (Fig. 1 a). When infiltrates were identified in relation to islets (an uncommon occur-

rence), the involvement of islets was focal, contiguous to the affected ductule, and/or venule (Fig. 1b). Envelopment and extensive permeation of islets by mononuclear cells was not noted. Aldehyde fuchsin-stained sections revealed a normal complement of β cells in islets (Fig. 1c), even those contiguous to ductular and/or venular cellular infiltrates.

Pancreatic immunoreactive insulin measurements

Pancreatic IRI concentrations varied widely in both the NZB and the control strains, but all animals had levels above those consistently found in overtly diabetic rodents. The mean concentrations were higher in the NZB mice (294.7 ± 27.2 ng/mg) than in the BALB/cJ (190.4 ± 45.1 ng/mg) and the CBA groups (261.9 ± 20.6 ng/mg), but the differences did not achieve statistical significance. Within the NZB group, the mean value in those animals in which pancreatic infiltrations were noted was higher than in animals with normal histology (317.7 ± 51.4 versus 280.5 ± 29.7 ng/mg), but again, the difference was not statistically significant.

Plasma glucose measurements

There was no difference in the mean value of plasma glucose between the BALB/cJ (5.3 ± 0.3 mmol/l) and the NZB (5.7 ± 0.3 mmol/l) groups; values were somewhat higher in the CBA strain (8.3 ± 0.2 mmol/l). No significant differences were noted between the NZB animals with pancreatic lesions (5.96 mmol/l) and those with a normal pancreas (5.59 mmol/l). Pancreatic histology, pancreatic IRI concentrations, and plasma glucose concentrations are presented in Table 1.

Table 1. Pancreatic histology, pancreatic IRI and plasma glucose concentrations in control and experimental NZB/BINJ mice

Strain	Age (weeks)	Sex	Pancreatic histology	Pancreatic IRI (ng/mg wet weight)	Plasma glucose (mmol/l)
Control mice					
BALB/cJ	27	M	Normal	236.6	5.3
($n=6$)	23	M	Normal	380.8	4.8
	19	M	Normal	194.8	4.1
	19	F	Normal	65.6	6.4
	15	F	Normal	53.5	4.8
	15	M	Normal	211.2	6.4
Mean ± SEM				190.4 ± 45.1	5.3 ± 0.3
CBA	28	F	Normal	162.3	7.6
($n=10$)	28	F	Normal	208.4	8.2
	28	F	Normal	205.5	8.0
	28	F	Normal	283.2	8.6
	28	F	Normal	205.3	8.9
	28	M	Normal	376.3	7.6
	28	M	Normal	260.1	7.9
	28	M	Normal	279.6	9.4
	28	M	Normal	356.2	7.4
	28	M	Normal	282.4	8.9
Mean ± SEM				261.9 ± 20.6	8.3 ± 0.2
Experimental mice					
NZB/	31	M	Normal	337.2	3.9
BINJ	31	M	One focus PVI	194.8	5.4
($n=21$)	31	F	Two foci PDI	276.7	6.2
	27	F	One focus PDI	259.5	5.3
	27	M	Normal	225.6	6.3
	27	M	One focus PDI with partial involvement of islet	266.7	6.0
	27	M	Normal	176.8	6.7
	27	F	Normal	391.0	5.9
	27	F	Normal	202.3	6.7
	23	M	One massive focus PDI with partial islet involvement	679.6	6.3
	23	F	One focus PDI	229.7	6.2
	23	F	Normal	266.3	3.1
	23	M	One focus PDI	380.4	4.9
	23	M	Normal	304.7	6.4
	23	F	Normal	424.2	6.4
	19	F	One focus PDI	254.1	7.4
	19	F	Normal	335.2	7.5
	19	F	Normal	222.9	5.9
	19	M	Normal	177.0	4.0
	19	M	Normal	97.7	2.6
	19	M	Normal	485.5	7.3
Mean ± SEM				294.7 ± 27.2	5.7 ± 0.3

PDI = periductal or periductular mononuclear cell infiltration
PVI = perivascular mononuclear cell infiltration

Discussion

This study demonstrates that autoimmune-prone NZB mice develop diffuse mononuclear cell infiltrates in a variety of tissues including lung, liver, salivary gland, mesentery, and pancreas. In the latter site, the infiltrates are centred in fibrous septae about ducts, ductules, and venules. Rarely, islets may be affected, but then, involvement is focal, related to that portion of the islet contiguous to affected ducts, ductules, and/or venules. The pattern of insulitis in human [13] and experimental [14–16] insulin-dependent diabetes mellitus characterized by mononuclear cell envelopment and extensive penetration of islets was not noted in this study. Moreover, histochemical stains for pancreatic β cells and radioimmunoassay determinations of pancreatic insulin content suggest that no major immunological assault is directed towards β cells.

The involvement of ductular and venular pancreatic structures in NZB mice is not dissimilar from that described experimentally in mice following the administration of anti-insulin serum [17] and after the induction of acute graft-versus-host reactions [18]. In graft-versus-host systems, both acute and chronic reactions result in the apparent homing of mononuclear cells to a variety of lymphoid and non-lymphoid targets. Depending on the system employed, epithelial injury is seen in the skin, gut, thymus, bronchi, and ductules of various tissues including liver, salivary gland, breast, and skin [19]. Others have described that acute graft-versus-host reactions may result in cellular immune reactions against islets with resultant β cell necrosis [20]. In our experience, pancreatic infiltration appears largely related to ducts,

ductules, and venules, with little direct involvement of islets. During the reaction, Ia antigens are expressed on certain epithelial cells which are normally negative for this antigen [21]. It is postulated, though not yet proven, that these newly expressed Ia antigens on target cells may serve as recognition units for effector cells responsible for tissue injury during the graft-versus-host reaction [19]. Whether a similar mechanism accounts for the distribution of mononuclear cell infiltrates in NZB mice is unknown.

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