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Mast Cells in the Islets of Langerhans in Insular Amyloidosis

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Summary. The number of mast cells in the pancreatic islets in persons 60 years or more without diabetes and with maturity-onset diabetes was determined in an autopsy study. Few mast cells were seen in sections of many islets without amyloid. In the presence of insular amyloidosis the number of intrainsular mast cells increased and this increase was positively correlated with to the amount of amyloid in the islets, both in diabetics and non-diabetics. It is conceivable that the mast cells are of some importance in the formation of amyloid in the pancreatic islets, but other explanations for the numerical increase in mast cells are possible.

Zusammenfassung. In einer Autopsieuntersuchung wurde die Anzahl von Mastzellen in den Pankreasinseln von 60jährigen und älteren Personen ohne Diabetes mellitus und mit Altersdiabetes bestimmt. Einige wenige Mastzellen wurden in Regionen vieler Inseln ohne Amyloid beobachtet. Bei Vorliegen von Amyloidosis war die Anzahl intrainsulärer Mastzellen erhöht, wobei diese Erhöhung eine positive Korrelation zur Menge Amyloid in den Inseln aufwies, sowohl in Diabetikern als auch Nicht-Diabetikern. Es ist denkbar, daß die Mastzellen eine Rolle im Zusammenhang mit der Amyloidbildung in den Pankreasinseln spielen, jedoch sind andere Ursachen für die zahlenmäßige Erhöhung der Mastzellen nicht auszuschließen.

Hyalinization of the islets of Langerhans is seen mainly in persons with maturity onset diabetes, but also in older persons without diabetes (Bell, 1952, 1959). Ever since its discovery (Opie, 1900), the nature of the hyaline has been subject to discussion. Since it was shown that hyaline had staining reactions typical for amyloid, especially with Congo red (Ehrlich and Ratner, 1961) and, further, displayed the same fine-fibrillar ultrastructure as amyloid in other locations (Lacy, 1964), the amyloid nature of hyaline seems to have been fairly generally accepted.

It is considered that amyloid is produced at the site of deposition, probably by reticulo-endothelial cells (cf. Ranløv and Wanstrup, 1968). In experimental amyloidosis induced with the aid of casein, findings have been made which strongly support this theory (Teilum, 1964).

The types of cell in the pancreatic islets which are most probably producers of amyloid are endothelial and connective tissue cells. In studies of amyloid in the islets of Langerhans I have had the impression that the number of mast cells is increased in association with amyloid deposition. The present study was con-

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ducted with the aim of finding out whether there is any difference between cases with and those without insular amyloidosis, with respect to the number of intrainsular mast cells in the pancreas.

Material and Methods

The study was carried out on autopsy material. Patients for examination were selected before the autopsy. Only persons 60 years old and older were included in the material.

All patients had been investigated in hospital. All those without diabetes mellitus had had several urine tests with a glucosefree result and in most cases at least one blood glucose determination with a normal result. In one of the patients diabetes had also been excluded by means of a glucose tolerance test. The diabetes material comprised only cases of clinically manifest maturity onset diabetes.

The total material consisted of 23 patients, of whom 11 were maturity onset diabetics (see Tables 1 and 2). The mean age of the diabetic patients was 71 years and that of the non-diabetic patients slightly higher, 76 years.

The time between death and autopsy varied between 4 and 11 hours. During the interval the bodies were kept at $+4°$ C. At autopsy the pancreas was dissected free as quickly as possible, and small pieces from the tail were placed in a fixative solution.

The following fixatives were used: Formalin, 10% (v/v); Buffered, neutral formalin, 10% (v/v); Isotonic formaldehyde-acetic acid mixture ("IFAA": Enerbäck, 1966a); A mixture of equal parts of 10% formalin (diluted with CO_2-free water) and 4% (w/v) lead subacetate (Pearse, 1960).

Sections of pancreatic pieces fixed in formalin and in buffered, neutral formalin were stained with thioflavine S (Schwartz, 1965) and alkaline Congo red (Puchtler *et al.*, 1962) for demonstration of amyloid.

For qualitative mast cell studies, formalin- lead subacetate- and IFAA-fixed material was used. After deparaffinization the sections were stained with 0.5% (w/v) toluidine blue at pH 4 and 0.5 (Enerbäck, 1966b) and with Alcian blue 8 GX at pH 0.5 (Bower and Chadwin, 1966). Alcian blue stained sections were counterstained with nuclear fast red.

Quantitative Methods

The quantitative studies were made on sections from pancreatic tail fixed in the formalin—lead subacetate mixture. After deparaffinization of 4 μ thick sections, these were stained with Alcian blue, as described above, and counterstained with van Gieson's stain and haematoxylin according to Mayer. An adjacent section was stained with alkaline Congo red.

For orientation concerning the distribution of the amyloid, the Congo red stained section was used. The Alcian blue-van Gieson stained section was magnified by means of a Leitz microprojector via a mirror. At a magnification of $\times$ 585 profile drawings of all islets in the section were made on paper, and the amyloid areas were marked in as carefully as possible. At the same time as the drawings were made the number of mast cells in the islets were counted. Only mast cells with a nucleus in the section were included in the count. Mast cells lying at the borderline between an islet and the surrounding tissue were counted only if they lay inside the basement membrane of the islet. The number of islets studied in this way was at least 75 per person. Finally, a profile drawing of the whole section was made at a low magnification ($\times$ 30.5), only exocrine and endocrine parenchyma being included.

The areas drawn were measured planimetrically. In each case a calculation was then made both of the degree of amyloidosis (i.e. the percentage of the total islet area occupied by amyloid) and of the number of intrainsular mast cells per mm^2 of the total islet area.

The mast cells in the exocrine parenchyma were counted in a microscope at a magnification of $\times$ 500. The diameter of the visual field at this magnification was 0.312 mm. 150 randomly chosen visual fields were studied in each section. From the values obtained in this way and previously obtained values for the amount of islet tissue and the number of mast cells in the islets, the true value for the number of mast cells in the exocrine parenchyma could then be calculated.

The sections were also studied with respect to fibrosis in the exocrine parenchyma and in the islets.

Results

Qualitative Studies

Exocrine Tissue. In the interacinar connective tissue sporadic mast cells were found. These cells were of different sizes and varied greatly in shape. Large, round mast cells with densely packed, large granules were commonly seen. A few mast cells were small and displayed fine, weak granulation reminiscent of that which is usual in intrainsular mast cells (vide infra). The mast cell granules showed deep metachromatic staining with toluidine blue at pH 0.5 and 4, and a strong affinity for Alcian blue.

Islets without Amyloid. In islets without amyloid in both diabetic and non-diabetic patients, one or more mast cells were found in many sections. These mast cells lay inside the islets in the immediate vicinity of blood vessels. They were relatively small and usually showed more sparse and finer granulation than mast cells in other parts of the pancreas. The staining properties were otherwise the same as in other mast cells. Mast cells on the borderline between islets and exocrine tissue were generally narrow and fibrocyte-like.

Islets with Amyloid. With thioflavine S and alkaline Congo red, hyaline in islets of both diabetics and non-diabetics showed staining properties typical for amyloid. The amyloid lay in the islets in a manner as has been described previously (cf. Warren *et al.*, 1966).

In islets with amyloid deposits mast cells were frequent and often lay in the immediate vicinity of amyloid. The shape of the mast cells varied more than in islets without amyloid and elongated, irregularly shaped mast cells were common. The mast cells were often sparsely granulated but the granules had the same staining properties as in pancreatic mast cells in general. No difference between the mast cells in hyalinized islets in patients with and in those without diabetes was found.

Quantitative Studies

The principal results are presented in Tables 1 and 2 and Fig. 1. Amyloidosis of different degrees in pancreatic islets was found in 7 (58%) of the patients without diabetes. Of the 11 diabetic patients 10 (91%) showed varying degrees of insular amyloidosis. In cases where very mild amyloidosis was found ($< 2\%$ of the islet area), only a very small number of islets were affected in each section. In these cases the islets with amyloid changes were localized to one or only a few places in the section.

Mast Cells. In the 5 non-diabetic patients without amyloidosis of the islets, the intrainsular mast cell count was 19.1 ± 3.0 per mm^2 (mean value $\pm$ SE), while 6 non-diabetic patients with mild islet amyloidosis ($< 2\%$ of the islet area) had 31.6 ± 3.3 intrainsular mast cells per mm^2. This difference is significant ($P < 0.02$). Only one of the non-diabetic patients showed pronounced insular amyloidosis (20.5%).

The diabetic patients showed a considerably more varying degree of amyloidosis of the islets than the non-diabetics. With an increasing degree of amyloidosis there

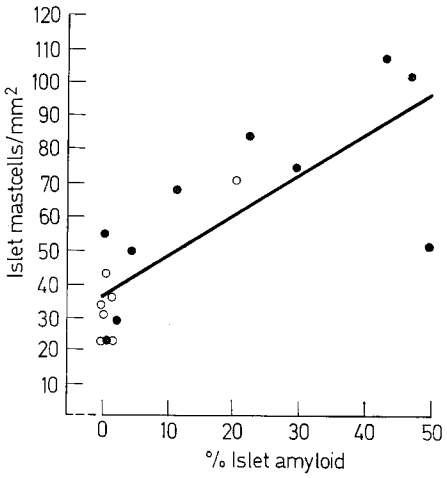

Fig. 1. Correlation between the degree of amyloidosis in the pancreatic islets and the number of intrainsular mast cells in diabetic (•) and non-diabetic (o) persons ($r = 0.79$; $Y = 1.2x + 36.4$)

Table 1. *Diabetic persons*

Case	Sex	Age at death (years)	Age at onset of diabetes (years)	Duration of diabetes (years)	Islet amyloid in % of islet area	Islet mast cells per mm² islet	Interacinar mast cells per mm² exocrine tissue
1	♂	72	66	6	11.4	68.6	33.5
2	♂	67	54	13	0.9	22.4	9.8
3	♂	69	63	6	43.1	108.0	11.3
4	♀	76	63	13	29.8	74.4	15.6
5	♀	66	55	11	4.8	50.0	33.9
6	♂	77	73	4	2.6	29.0	5.1
7	♀	68	64	4	0.0	33.1	14.2
8	♀	76	65	11	22.9	83.9	24.1
9	♀	71	49	22	49.5	51.2	16.7
10	♂	65	62	3	0.6	55.0	13.9
11	♀	78	73	5	46.8	102.3	30.8

was a distinct tendency to an increased number of intrainsular mast cells, and this increase is significant ($r = 0.70$; $P < 0.05$).

Fig. 1 shows the relationship between degree of amyloidosis and number of intrainsular mast cells in diabetic and non-diabetic patients. A clear, statistically significant positive correlation was found ($r = 0.79$; $P < 0.001$).

The interacinar mast cells showed no correlation with amyloidosis of the islets, either in diabetics, non-diabetics or both these groups combined. No statistically significant difference was found in the number of interacinar mast

Table 2. *Non-diabetic persons*

Case	Sex	Age at death (years)	Islet amyloid in % of islet area	Islet mast cells per mm² islet	Interacinar mast cells per mm² exocrine tissue
1	♀	87	0.0	22.3	15.8
2	♀	69	0.0	14.6	[a]
3	♂	84	0.0	28.8	19.6
4	♂	67	0.3	33.7	14.3
5	♀	83	1.5	22.8	8.2
6	♀	84	0.6	31.2	19.5
7	♀	78	0.0	17.9	9.0
8	♀	86	0.0	12.1	16.2
9	♂	71	20.5	70.7	9.9
10	♂	77	0.9	43.3	25.7
11	♂	60	0.2	22.7	26.4
12	♀	67	1.8	36.1	19.5

[a] The number of interacinar mast cells was not measured in this case because of a severe pancreatic lipomatosis.

cells between the diabetic and the non-diabetic group. On the whole the number of interacinar mast cells varied much less than the number of intrainsular mast cells.

Discussion

The morphology of the islets of Langerhans has been the object of many, comprehensive investigations. Little interest has been paid, however, to the interstitial islet cells.

Mast cells occur in most parts of the body, but almost exclusively in connective tissue (Selye, 1965). The number of mast cells varies considerably from place to place. Only sparse information is given in the literature on the occurence of mast cells in the pancreas. These cells are reported to occur in the pancreatic stoma in the guinea pig (Zimmermann, 1908), the dog (Arvy and Quivy, 1955), the hamster (Kelsall and Crabb, 1959) and in man (Staemmler, 1921). In the hamster occasional mast cells occur normally close to afferent blood vessels of the islets (Kelsall and Crabb, 1959).

A local increase in the mast cell density is seen in different pathological conditions (cf. Selye, 1965). Some reports have also been made on an abundant occurence of mast cells in association with amyloidosis (Christensen, 1968; Brini and Porte, 1970) and in amyloid stroma in insuloma (Steiner, 1969). No quantitative analysis of the occurence of mast cells in amyloidosis appears to have been reported previously.

From the present study it was evident that mast cells are normally found in the vicinity of blood vessels in the pancreatic islets in man. Mast cells were not seen in all islet sections, however. It was also evident that the intrainsular mast

cells were more numerous in cases of islet amyloidosis and that the mast cell density increased with increasing degree of amyloidosis.

The mast cells in the exocrine part of the pancreas showed no tendency to increase in amyloidosis in the islets of Langerhans, and the intrainsular mast cell increase is thus no consequence of a general mast cell increase in the pancreas.

Mast cells are always present in new formation of connective tissue (Asboe-Hansen, 1968). One possible reason for the numerical increase in intrainsular mast cells in amyloidosis of the islets might be the presence of fibrosis in the islets parallel with the amyloidosis. Insular fibrosis and insular amyloidosis are sometimes seen in the same pancreas and even in the same islets, but this is not the rule (Ogilvie, 1964). No parallelism between fibrosis in islets or in the exocrine parenchyma, and insular amyloidosis was found in this study. Fibrosis in both islets and exocrine parenchyma was somewhat more common in pancreas from diabetic patients than in that from non-diabetics, however.

The cause of the increase in intrainsular mast cells in amyloidosis of the islets is thus unclear. One possibility is that mast cells take part in amyloid production. It is also conceivable that the increase in mast cells is a result of the amyloid deposition, or that it is only an associated phenomenon.

Of the patients with insular amyloidosis the value for one lay far outside the regression line (Fig. 1). This patient, who showed an unusually high degree of amyloidosis (49.5%), had the longest duration of diabetes in the group (22 years). It is conceivable that the number of mast cells only increases during the course of amyloid formation, and that this latter is not a continuous process but diminishes when the islet tissue begins to decrease.

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