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A morphological analysis endocrine tumour genesis in pancreas and anterior pituitary of AVP/SV40 transgenic mice

G. Rindi[1], A.E. Bishop[1], D. Murphy[2], E. Solcia[4], B. Hogan[3], and J.M. Polak[1]

[1] Department of Histochemistry, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 OHS, UK
[2] Unit of Veterinary Cellular and Molecular Biology, Royal Veterinary College, London UK
[3] Laboratory of Molecular Embryology, National Institute of Medical Research, London, UK
[4] Department of Human Pathology, University of Pavia, Italy

Summary. Insertion into the mouse genome of the hybrid oncogene made up of bovine vasopressin gene derived 5′ upstream sequences and the coding sequences of SV40 large T-antigen promoted tumours in anterior pituitary and endocrine pancreas of mice bearing this transgene. In order to investigate the morphology of the steps in the neoplastic process, we used light and electron microscopy to study these organs in 42 animals belonging to the 3rd, 4th and 5th generations, subdivided into 4 age groups from 20 days to 100 days of life. Antibodies to large T-antigen were used to identify sites of expression of the hybrid oncogene, thus monitoring the steps in neoplastic transformation. Large T-antigen immunoreactivity was identified in dysplastic lesions of younger animals and in both dysplastic lesions and tumours of older mice. Insulin (100% of cases) and pancreatic polypeptide (25% of cases) immunoreactivities were revealed in pancreatic lesions but no hormonal immunoreactivity was detected in the pituitary lesions. The ultrastructural study confirmed that the majority cell population of the pancreatic neoplasms was B-type and that the anterior pituitary tumours were poorly granulated. The subcellular localization of large T-antigen immunoreactivity was investigated by the immunogold method and was confined to the heterochromatin of tumour cell nuclei. These findings provide evidence for the dysplasia-neoplasia sequence in the genesis of endocrine tumours of pituitary and pancreas of transgenic mice. The vasopressin-SV40 large T-antigen transgenic mice may therefore be an useful model for the study of endocrine cell oncogenesis.

Offprint requests to: J.M. Polak

Key words: AVP/SV40 transgenic mice – Immunocytochemistry – Insulin – Pancreatic polypeptide – Large T-antigen – Heterochromatin – Dysplasia – Neoplasia

Introduction

Insertion into the mouse genome of the viral sequences of Simian Virus 40 (SV40) encoding for the transforming protein large T-antigen, proved to be oncogenic either when its expression was under the control of its natural viral promoter (Brinster et al. 1984) or that of a novel promoter as a part of a hybrid oncogene (Hanahan 1985; Ornitz et al. 1985; Power et al. 1987). A transgene comprising the putative regulatory sequence of the bovine arginine-vasopressin (AVP) gene and the sequences encoding SV40 large T-antigen, has been shown to promote tumour genesis in the endocrine pancreas and anterior pituitary of transgenic mice (Murphy et al. 1987). Six founder animals (VT A-F) were derived and one (VT-C) generated a stable transgenic mouse line. The first generation of VT-C animals died prematurely, around 90 days of life, and had pancreatic B-cell tumours and poorly granulated endocrine tumours of the anterior pituitary (Murphy et al. 1987).

As large T-antigen can be detected by immunocytochemistry (Hanahan 1985; Adams et al. 1987; Murphy et al. 1987), we hypothesised that, by following the production of this antigen in target organs of AVP/SV40 transgenic mice, in combination with conventional histology and immunocytochemistry of endocrine cell products, it would be

possible to study in detail the various morphological stages of the oncological process.

In human pathology, dysplasia accompanies many neoplasms and, when of a high grade, is thought to precede the growing of true neoplasia. Nevertheless, morphological evidence that dysplasia evolves to neoplasia is mostly only indirect or, in some cases, completely lacking. In view of this, we further hypothesised that, by studying the expression of large T-antigen in dysplastic lesions as well as in tumours of target organs in AVP/SV40 transgenic mice we could derive experimental evidence of the supposed link between the two pathological states. Initially, attention was focused on VT-C transgenic mice older than 90 days, at which time the oncogenic effects of the AVP/SV40 transgene were most prevalent. Following characterisation of the different transforming steps of the process, the time course of oncogenesis was examined by investigating animals at different ages.

Materials and methods

AVP/SV40 transgenic mice of the VT-C line ($n=42$) and wild type controls ($n=8$) were sacrificed by ether inhalation and underwent a complete autopsy. The animals were divided into 4 age groups: 90–100 days old ($n=28$), 50–60 days old ($n=5$), 40–50 days old ($n=4$) and 20–30 days old ($n=5$). Whole pancreas and pituitary were removed and fixed in Bouin's fluid for 4 h at room temperature. Wax blocks were cut serially at 3 μm thickness. The sections were stained with haematoxylin and eosin for conventional histology and immunostained for large T-antigen and a range of different endocrine cell products (see Table 1) using the peroxidase anti-peroxidase (PAP) method (Sternberger 1979) or the streptavidin-biotinylated peroxidase complex (SABC, Amersham International, UK) method (Hsu et al. 1981). Before immunostaining, some sections were treated with trypsin (Sigma Chemicals, Poole, UK) (0.03% in 0.1 M phosphate buffered 0.15 M saline, PBS, pH 7.4) or subtilisin BPN nagarse (protease type XXVII, Sigma Chemicals, Poole, UK) (0.003% in PBS, pH 7.4) for 5 to 15 minutes at room temperature (Fiocca et al. 1987).

For ultrastructural studies, small samples from pancreatic ($n=4$) and pituitary ($n=2$) tumours were fixed by immersion in Karnovsky's fluid (Karnovsky 1965) or in glutaraldehyde (2% in 0.1 M phosphate buffer, pH 7.4) for 2 h at 4° C. For conventional electron microscopy, tissue was further postfixed in osmium tetroxide (1% in 0.1 M phosphate buffer, pH 7.4) for 1 h at 4° C. The specimens were then processed into Araldite. Semithin sections (0.5–1 μm) were cut from osmicated tissue and stained with toluidine blue (1% in 3% aqueous borax). Areas of interest were trimmed and then sectioned (60–100 nm) with a Reichert Ultracut E ultramicrotome. Ultrathin sections were collected on uncoated nickel 200 mesh grids, desiccated, counterstained with uranyl acetate and Reynold's lead citrate and then observed in a Zeiss 10 CR transmission electron microscope. Semi-thin sections (0.5–1 μm) from non-osmicated tissue were also immunostained with PAP method in order to assess by light microscopy the preservation of large T-antigen after resin embedding. The immunogold staining of large T-antigen was performed using gold labelled IgG (Janssen Pharmaceutica, Beerse, Belgium) as described by Varndell et al. (1982).

Table 1. Antisera used in this study

Antiserum to	Optimal dilution	Source
Large T-antigen	1:200	D. Lane, Imperial Cancer Res. Fund, UK
Insulin	1:200	Miles Res. Products Ltd., UK
Glucagon	1:5000	Hammersmith Hospital, UK
Pancreatic polypeptide	1:1000	R. Chance, USA
Somatostatin	1:10000	RIA, UK Ltd., UK
LH	1:4000	A.F. Parlow, USA
FSH	1:1000	A.F. Parlow, USA
GH	1:8000	Wellcome Reagents Ltd., UK
MSH	1:2500	H. Vaudry, France
TSH	1:2000	A.F. Parlow, USA
Prolactin	1:1000	Wellcome Reagents Ltd., UK
ACTH	1:2000	Wellcome Reagents Ltd., UK

All antisera were raised in rabbits except for the anti-insulin which was derived from guinea pig

Specificity tests for the immunostains consisted of absorption of each antiserum with its homologous antigen (10 nmol/ml), omission of the first layer and use of control tissue with or without the pertinent antigen (Van Noorden 1986). Absorption of the antiserum against large T-antigen with low molecular weight poly-L-lysine (Sigma Chemicals, Poole, UK) was performed as previously reported (Scopsi et al. 1986).

Results

Age 90–100 days

This group was composed of 14 mice of each sex (mean weight males 30 g; females 24 g). Of these animals, 71% ($n=20$) belonged to the third generation and 17% ($n=5$) and 12% ($n=3$) to the fourth and fifth generations, respectively. Tumours of both pituitary and pancreas were present in 35% of animals ($n=10$), with a prevalence of females (70%, $n=7$).

Pancreas

Of the 28 pancreata examined at autopsy, 27 were studied microscopically. Tumours were detected in 20, dysplasia in 3 and 4 presented a completely normal appearance as compared with the controls. The case which was not studied histologically showed evident neoplasms at gross examination. The tumours were multiple, red-bluish, round masses of variable size (maximum diameter 0.6 cm), with a solid consistency and reddish cut surface. They were randomly distributed in the organ and no consistent location could be observed.

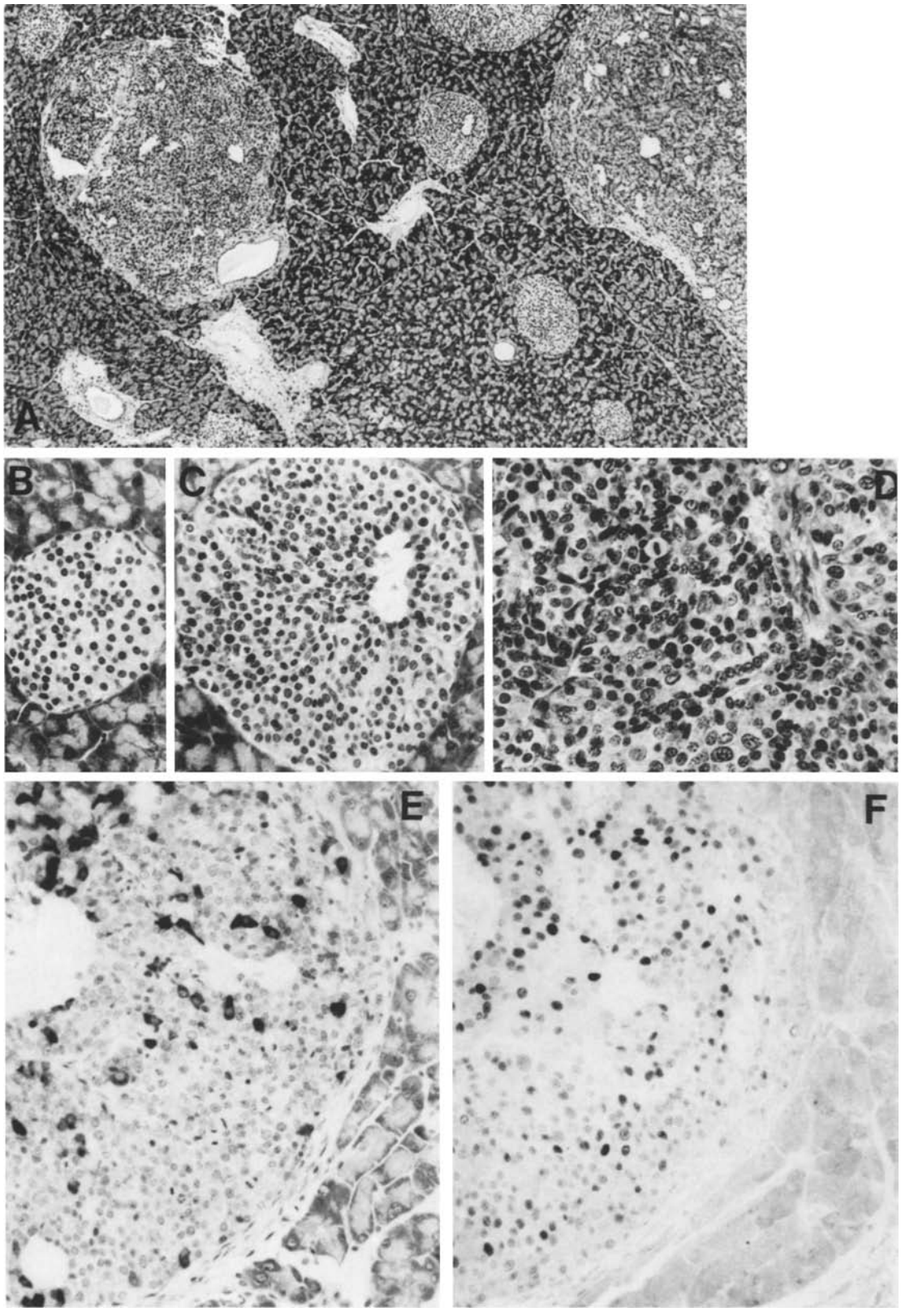

Fig. 1 A–D. 90–100 days old transgenic mouse pancreas. **A–D** H&E ×90 (**A**), ×340 (**B–D**). **A** General view of the organ with tumours, dysplastic and normal islets. **B** High power micrograph of normal islet. Compare the cytology with the following figures. **C** Dysplastic islet with crowded cells delimiting a lacunar space, nuclear polymetricism and spotted distribution of chromatin. Some cells appear to maintain a normal nucleus/cytoplasm ratio. **D** Detail of the cytology of the tumour in **A**, near a middle sized duct (*middle left margin*). Note the high mitotic ratio, the nuclear polymetricism and the prevalent spotted pattern of the chromatin. **E–F** PAP method, haematoxylin counterstain (**E**), ×400. **E** Pancreatic polypeptide-immunoreactive cells. **F** Large T-antigen-immunoreactive cells. Note the variability of intensity of the immunoreaction and the absence of any signal in the neighbouring exocrine elements

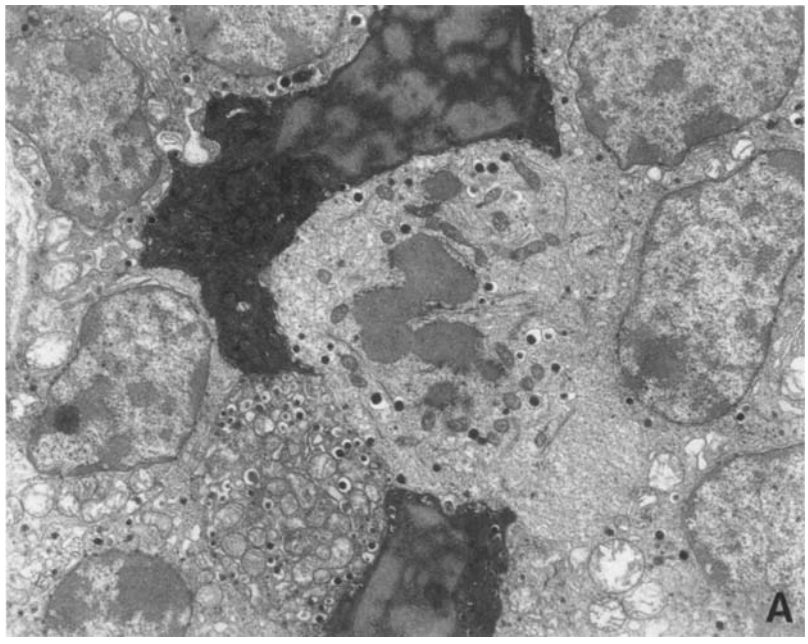

Fig. 2. Tumour from 90–100 days old transgenic mouse pancreas. General view of the ultrastructure of the neoplastic elements. Note the irregular mitosis in the middle and the variable distribution of granules in the cells (×8800)

The remaining parts of the organ had a normal macroscopic appearance.

The histology of the 27 pancreata examined revealed pathological findings in 23. These cases showed variable increases in the endocrine comparment of the organ (Fig. 1A–D), partly due to full blown neoplasms, partly to irregularly enlarged islets containing dysplastic areas and partly to cytologically normal islets often showing spatial relationships with middle-sized and small ducts. The budding of groups of endocrine cells from ducts was observed frequently. Dysplasia was defined as *foci* of endocrine cells with nuclear atypism organised in a solid, diffuse structure inside an otherwise normal islet. The dysplastic islets were multiple and contained nests of crowded cells, sometimes with delimiting lacunar spaces and with modified nuclear/cytoplasmic ratios, irregular nuclear size with a spotted distribution of chromatin, prominent nucleoli and, sometimes, mitoses. Note that 3 cases out of 23 did not reveal neoplasms, although they displayed the other pathological features. The tumours were multiple, large growths, not encapsulated, with margins pushing off the surrounding exocrine parenchyma without infiltration. The structure of the tumours varied from the most frequent solid pattern with lacunar spaces, to a gyriform or ribbon-like pattern. Individual tumour cells had variable amounts of granular eosinophilic cytoplasm with an increased nuclear/cytoplasmic ratio. Nuclei were polymorphic, sometimes very large and bizarre, frequently with prominent nucleoli and always with a characteristic spotted chromatin closely resembling that of the dysplastic cells. Moreover, frequent, often atypical, mitoses were observed. The stroma varied in abundance and was richly vascularized in most cases.

In the remaining parts of these organs no ductal proliferation nor increase in the fibrotic stroma could be observed. In a single case a moderate lympho-monocytic infiltrate was observed near and partly around tumours and dysplastic lesions. In the normal islets, the immunostains revealed the expected proportions of B, A, PP and D cells (Orci 1981; Klöppel and Lewson 1984). The same hormone immunoreactivities were observed in the neoforming islets near the ducts, while some scattered single elements immunoreactive for insulin or glucagon and, less frequently, PP or somatostatin, were found in the ductular epithelium or in small nests in the connective tissue surrounding the duct. The islets containing dysplastic areas revealed a variable pattern of intensity of immunoreactivity for insulin in the nests of dysplasia, while the remaining parts of the islets showed the usual characteristic density and distribution of other pancreatic endocrine cell types. The tumours were composed of insulin-immunoreactive elements with an extremely variable pattern of intensity of immunoreaction. Some scattered cells positive for glucagon, pancreatic polypeptide or somatostatin could be seen, mostly at the margins of the lesions where remnants of the normal insular structure could be observed sometimes. Five out of 20 cases

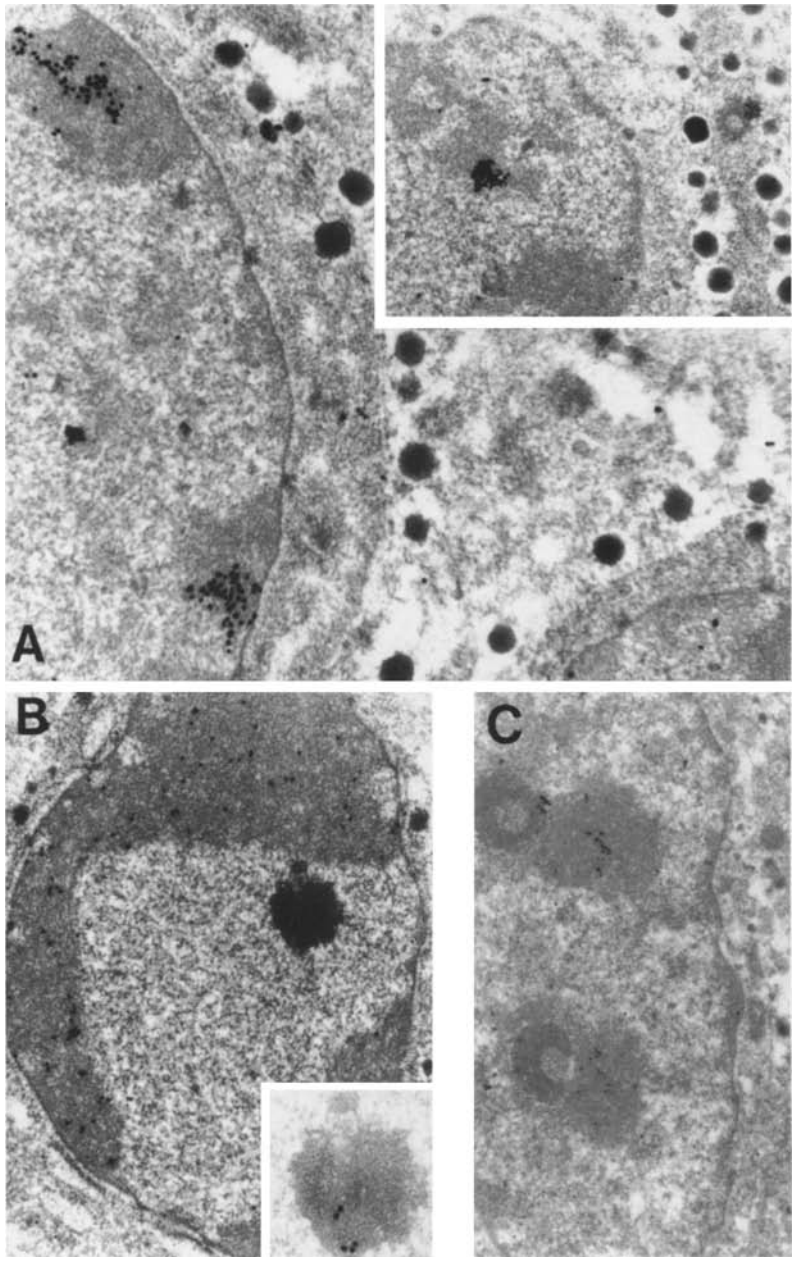

Fig. 3. A–C Tumour from 90–100 days old transgenic mouse pancreas. Indirect immunogold labelling of large T-antigen, gold particles 20 nm (**A–B**) and 15 nm (**C**). **A** ×30400, insert ×16000. Note the distribution of gold particles in the heterochromatin. **B** ×22400, insert ×35200 and **C** ×24000. The gold labelling is limited to the heterochromatin (**B**) and near the nucleoli (insert and **C**)

showed a mixed endocrine tumoural population, with a well developed PP-immunoreactive component (Fig. 1E).

Large T-antigen immunoreactivity was observed only in the cells showing cytological alterations, both in the dysplastic areas of small/enlarged islets and in the neoplasms (Fig. 1F). The immunoreaction was confined to the nucleus and varied in intensity. A significantly stronger large T-antigen immunoreaction could be seen in those cells which showed less insulin immunoreactivity in serial sections. No large T-antigen immunoreactivity was detected in cytologically normal islets including the endocrine cells budding off ducts. Absorption of large T-antigen antiserum with purified SV40 large T-antigen (10 nM/ml) prevented the immunostaining.

Electron microscopy

All the tumours ($n=4$) sampled for ultrastructural analysis were shown by light microscopy to consist mainly of B cells. Ultrastructurally, most cells showed irregular nuclei frequently with prominent

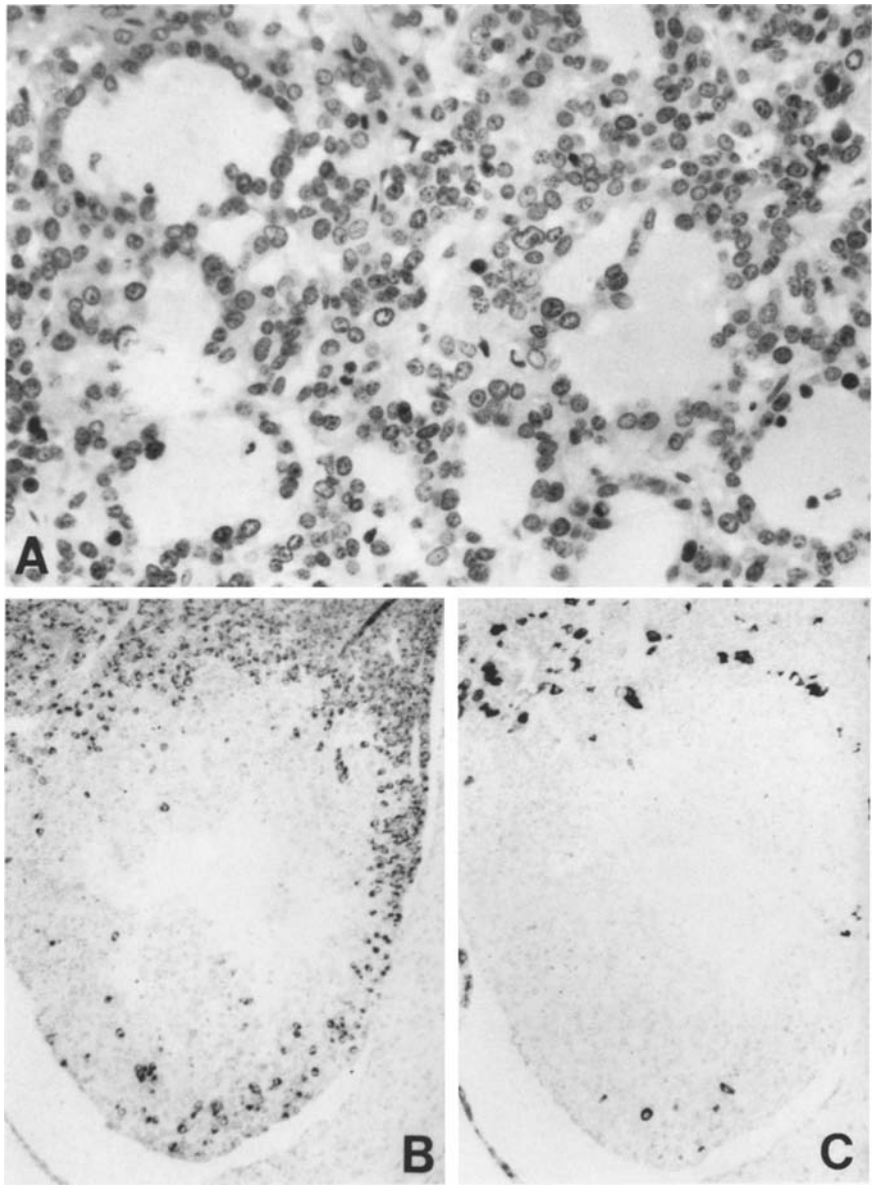

Fig. 4A–C. Tumours from 90–100 days old transgenic mouse pituitary. **A** H&E × 384. Structure and cytology of a large neoplasm. The lacunar spaces are delimited by single/double layers of elements and filled with plasma. The cells show a high degree of anaplasia with high mitotic rate. Note the spotted pattern of the chromatin. **B–C** PAP method, haematoxylin counterstain, serial sections × 200. Immunoreactivity for prolactin (**B**) and human chorionic gonadotropin (**C**). Small tumour occupying the anterior lobe of transgenic mouse pituitary. Unreactive tumoural cells are delimited by positive elements at the periphery. Note some immunoreactive cells, especially those containing prolactin (**B**), in the middle of the neoplasm which presents a mainly solid structure delimiting a central lacunar space. The borders of the neoplastic process are irregular

nucleoli, numerous mitochondria and abundant secretory granules (Fig. 2A–B). Some cells had fewer granules scattered between numerous, enlarged, round mitochondria (oncocytoid pattern) with swellings and loss of cristae. The granules (Fig. 2C) were round, mostly small (150–350 nm diameter) with a thin halo and a dense core, sometimes with geometrical shape. Many cells revealed multilamellar bodies in the cytoplasm (Fig. 2D). All these features indicated that these elements were mature, well granulated, insulin-producing B cells (Lacy 1961; Lacy 1962; Howell et al. 1969). After positive tests for large T-antigen on semithin sections, it was possible to identify in thin sections by the immunogold technique large T-antigen immunoreactivity in the heterochromatin of the nuclei of the above cells (Fig. 3A–C). Sometimes gold particles were observed in the heterochromatin

near the nucleoli (Fig. 3B insert -C). No significant labelling was noted in the euchromatin or in the cytoplasm.

Pituitary gland

Of 26 pituitary glands examined at autopsy, 23 were studied microscopically and the presence of tumours was revealed in 11. The neoplasms, when recognizable at gross examination, appeared as round, bluish masses (maximum diameter 0.5 cm) of highly vascularized soft, loose tissue, completely occupying the sella turcica. The microscopical examination of non-tumoral cases ($n=12$) revealed a completely normal structure in 9, as compared with controls, while in 3 cases high grade dysplasia was seen. These lesions were single in each organ and presented as small foci of atypical cells with modified nuclear/cytoplasmic ratio, irregular size and shape of nuclei with spotted chromatin, the tumoral pituitaries showed a completely disrupted architecture in 2 cases, while in the remaining 9 the growth was confined to part of the anterior ($n=8$) or intermediate ($n=1$) lobes. The larger tumours were composed of trabeculae of single or double layers of cells, delimiting lacunar spaces filled with cellular debris, plasma and blood elements (Fig. 4A). Some solid areas could also be seen. Small tumours showed a trabecular pattern with delimiting central lacunar spaces and solid areas at the periphery. Individual tumour cells were irregular in size and shape with scant eosinophilic cytoplasm and an increased nuclear/cytoplasmic ratio. Nuclei were polymorphic, sometimes bizarre, with dusty spotted chromatin. Frequent, often atypical mitoses could be seen. The remaining parts of the organ, when present, sometimes showed scattered abnormal cells with giant nuclei and isolated mitoses, between the normal cells. The immunostains revealed a normal pattern of endocrine cells (Nakane 1970) in the non-tumoral cases ($n=12$) and in the normal remnants of pituitary, when present, in the tumour-bearing glands (Fig. 4B–C). Dysplastic as well as tumoral cells showed no hormonal immunoreactivity although some scattered positive, normal appearing cells could be seen, especially at the periphery of the tumours.

Immunoreactivity for large T-antigen was demonstrated in the nuclei of dysplastic and neoplastic cells only (Fig. 5A–B). As with the pancreatic lesions, the intensity of the reaction was variable. No large T-antigen immunoreactivity was observed in the normal appearing remnants of the gland, in normal pituitaries or in the controls.

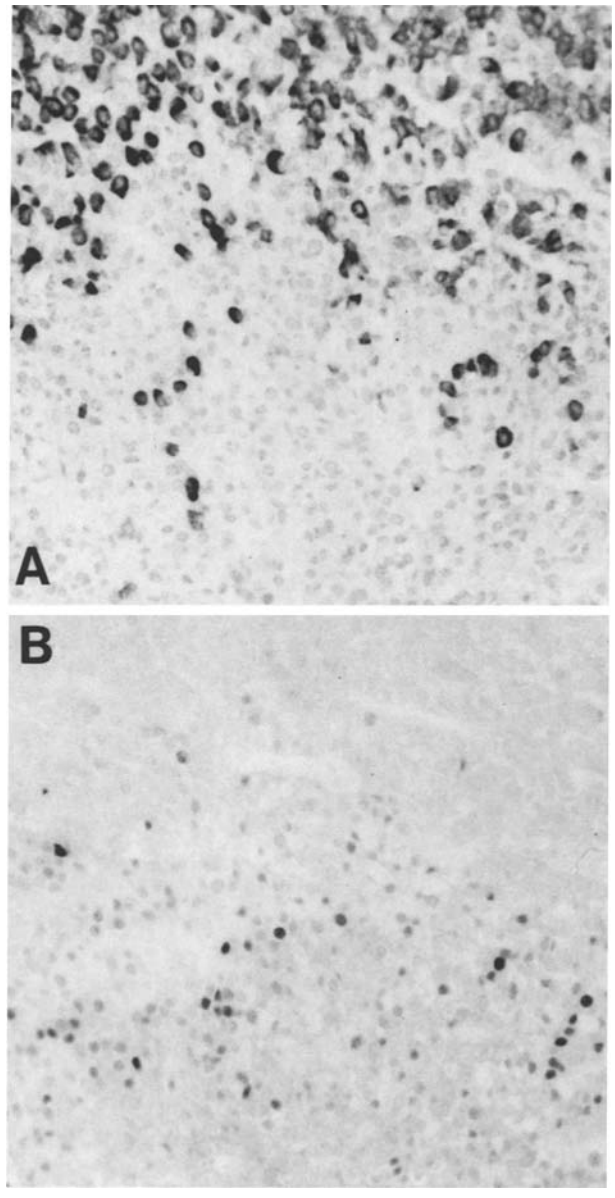

Fig. 5A, B. Tumour from 90–100 days old transgenic mouse pituitary. PAP method, haematoxylin counterstain (**A**), ×304. Immunoreactivity for prolactin (**A**) and large T-antigen (**B**). Large pituitary tumour showing irregular margins and normal prolactin-immunoreactive population (*upper side*). Note the presence of some positive elements between unreactive neoplastic cells. Large T-antigen immunoreactivity is confined to the neoplasm (lower side). Note the variable pattern of intensity of the nuclear immunoreaction and the presence of some faintly positive nuclei between negative cells at the border

Electron microscopy

The pituitary tumours studied ($n=2$) were composed of poorly granulated or even agranular cells. Most cells showed irregular nuclei with heterochromatin mostly located along the nuclear mem-

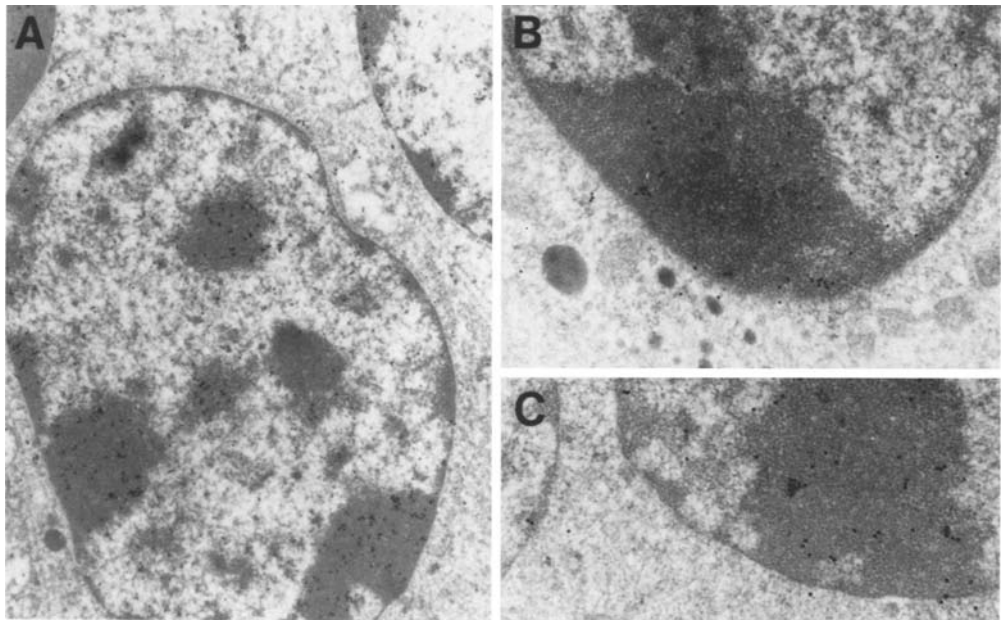

Fig. 6. A–C Tumour from 90–100 days old transgenic mouse pituitary. Indirect immunogold labelling for large T-antigen, 15 nm gold particles. A ×16000, B ×32000, C ×28800. The gold particles are localized to the heterochromatin. Note the presence of few, scattered endocrine granules (**A–B**)

brane, scant cytoplasm with well developed Golgi zones, abundant ergastoplasmic cisternae and numerous mitochondria. Whorls of ergastoplasmic cisternae were observed frequently. The granules were round, electron-dense and varied greatly in diameter (90 nm to 750 nm). The immunogold technique on thin sections allowed identification of large T-antigen immunoreactivity in the heterochromatin of the nuclei of the tumour cells (Fig. 6A–C). No significant labelling was noted in the euchromatin or in the cytoplasm.

Age 50–60 days

This group was composed of 5 animals: 4 male, 1 female (mean weight for both sexes 21.8 g). These mice all belonged to the third generation. None of them revealed any abnormality in pancreas, pituitary or any other organ at gross inspection. In one case, the histology of the pancreas revealed intrainsular high grade dysplasia accompanied by non-dysplastic endocrine budding off from ducts. In two other cases, groups of endocrine cells could be seen near ducts and ductules. The two other pancreata showed no difference from controls.

Immunoreactivities for pancreatic hormones showed a normal distribution in all animals. In the dysplastic lesions identified in a single case, uneven insulin immunoreactivity was demonstrated as well as nuclear large T-antigen immunoreactivity. Microscopical examination of the pituitaries ($n=5$) revealed no significant alteration nor any abnormal hormonal immunoreactivity or positive staining for large T-antigen.

Age 40–50 days

This group consisted of 4 animals (2 of each sex) (mean weight 18 g), all belonging to the third generation. None of them showed any gross pathology, while the histology revealed, in one case, high grade dysplasia in the pancreas (Fig. 7A). The normal pattern of pancreatic hormones was revealed by immunohistochemistry with irregular insulin immunoreactivity in the single dysplastic lesion together with nuclear large T-antigen immunoreactivity (Fig. 7B–C). In all but one animal the pancreas had a discrete endocrine proliferation near ducts and ductules (Fig. 7D–F). The microscopical examination of 3 pituitaries revealed no histological abnormality, a normal pattern of hormonal production and no large T-antigen immunoreactivity.

Age 20–30 days

This group was composed of 5 animals (3 females and 2 male; mean weight 12 g), belonging to the third generation. None of them showed any mac-

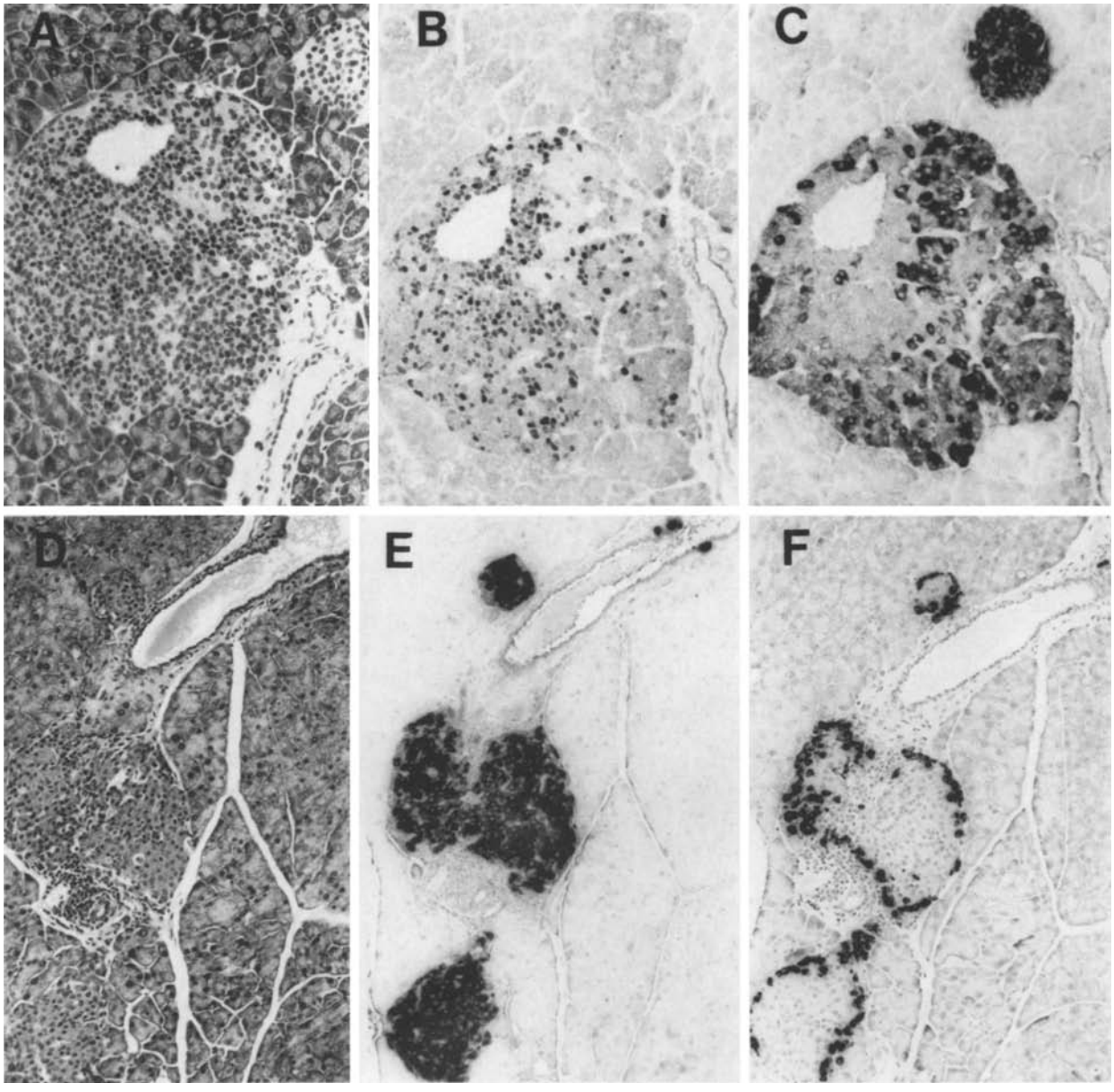

Fig. 7A–F. 40–50 days old transgenic mouse pancreas. **A–C** H&E (**A**), PAP method (**B–C**), no counterstain, serial sections, ×200. Dysplastic (centre) and normal (*top right*) islets near a middle sized duct. The large T-antigen immunoreactivity (**B**) is more intense in the cells surrounding the lacunar space while the insulin immunoreactivity (**C**) is stronger in the normal appearing part of the islet. Note the intense staining for insulin in the normal islet with no nuclear large T-antigen staining. **D–F** H&E (**D**), PAP method (**E–F**), haematoxylin counterstain, serial sections, ×140. Note the relationship between endocrine cells and ducts of large size (*upper right*) and small ductuli (*middle left*). Insulin-immunoreactive cells (*E*) are prevalent, with positive elements in the ductal wall (*upper right*). The glucagon-immunoreactive cells (**F**) maintain the usual distribution in the neoforming islets and are present in the duct epithelium (*upper left*)

roscopical lesion. At the microscopical level a discrete endocrine presence near ducts and ductuli with clear budding off could be seen in three pancreata, in one of which a high grade dysplasia was observed with a prevalent irregular insulin immunoreactivity. The remaining organs had a normal endocrine pattern. Nuclear large T-antigen immunoreactivity was observed only in the dysplastic lesion. Microscopical examination of the pituitaries ($n=5$) revealed no histological alteration, abnormal hormone production or large T-antigen immunoreactivity.

Discussion

In a previous study (Murphy et al. 1987), we described the lesions found in anterior pituitary and endocrine pancreas of AVP/SV40 large T-antigen transgenic mice. The pathological findings described in these first generation animals were full blown endocrine tumours in both organs, with some hyperplastic changes in the pancreas. The aim of the present study was to identify the morphological steps which we hypothesized preceded the appearance of overt neoplasms. To achieve this, we firstly studied a large number of animals at 90 days to 100 days of age, when there is a high level of expression of the AVP/SV40 large T-antigen transgene, thus establishing the major morphological criteria of the hybrid oncogene-induced lesions. Secondly, the time-course of the development of these lesions was assessed in samples from younger animals.

As in the initial study (Murphy et al. 1987), tumours were found in the endocrine pancreas and pituitary and the morphology observed in this study was comparable to that seen in the first generation of transgenic mice. In 25% of the pancreatic lesions examined in the present study (5 out of 20), a well developed PP-immunoreactive subpopulation of cells was seen. This was the only additional feature of significance found in the full blown lesions. Large T-antigen immunoreactivity was identified in the nuclei of all the tumour cells. Sometimes, it was possible to demonstrate an apparent inverse relationship between hormone immunoreactivity and the expression of large T-antigen. This suggested that the oncogenesis deranged the normal synthetic pathways inside the endocrine cell, progressively inducing a block to hormone production. Such an observation could explain the poor granularity of the tumour cells of pituitary. The regressive changes demonstrated at the ultrastructural level in some pancreatic tumour cells (oncocytoid pattern), could also support this hypothesis.

The above features characterised transgene expression in the neoplastic lesions and provided evidence that the production of large T-antigen was strictly associated with the neoplastic process. However, these features were also identified in lesions which did not fulfill the morphological criteria of tumours. These were found in the oldest group of animals both in pituitary (3 cases out of 23) and more frequently, in all the tumour-bearing pancreata (20 out of 27), and in 3 non-neoplastic cases. These lesions were interpreted as dysplastic, as they showed cytological atypia and structurally abnormal proliferative changes of the organ under study. In view of the severe cytological derangement observed, the dysplasia was classified as high-grade. The extreme similarity between the dysplastic and neoplastic cells, strongly suggested that they were elements undergoing a similar transforming process. The demonstration of large T-antigen immunoreactivity in the dysplastic as well as neoplastic nuclei supported this premise, which was also confirmed in the pancreas by the identification of most of these elements in both dysplastic and tumour lesions as B cells with disturbed insulin immunoreactivity. These data provided evidence that dysplasia was basically the morphological counterpart of the neoplastic transformation of cells when still inside an otherwise normal organ. This was considered to be an early step in the neoplastic process preceding the evolution of full blown neoplasia, which we took to be the uncontrolled, exaggerated proliferation of these dysplastic/transformed cells.

In the pancreas we observed endocrine cell proliferation from the ductular epithelium, with budding off and insular neogenesis. This phenomenon, constituted by cytologically normal as well as dysplastic cells showed that the endocrine pancreas was undergoing some proliferative change. The whole picture observed, with the presence of multiple full blown tumours, high grade dysplasia in islets and endocrine cell proliferation, could indicate that these pathological findings are different steps of an unique transforming process. The lack of detectable large T-antigen immunoreactivity in proliferating endocrine cells within ducts suggests that the proliferation was due to the production of trophic factor(s) by the tumour and dysplastic cells. This is a well known phenomenon (Larsson 1973) and could well be due to the metabolic effects of insulin (Porte and Halter 1981) produced by the proliferating B cell.

Large T-antigen-bearing lesions were not found in all animals and, moreover, only 50% of mice had lesions in both target organs simultaneously. In addition, we identified multiple hormone production in tumours of 5 out of 20 pancreata investigated. These findings suggested that, at least in pancreas, the neoplastic process was multicentric, as it involved separate endocrine structures at the same time, and could be polyclonal to a variable extent, as it affected, in the same tumour, different types of cells which had already differentiated to give their hormonal production. The fact that 70% of the animals presenting full blown neoplasms in both endocrine organs were female, suggested that factors specifically related to sex might have played a role in the genesis of tumours.

The data derived from the study of younger

animals (20–60 days old) indicated that transgene expression had started in the pancreas even during the very early days of life. Large T-antigen immunoreactivity was identified in dysplastic lesions in all 3 age groups. A common morphological pattern of endocrine neogenesis was also present in a high percentage of all 3 groups of animals, confirming that endocrine proliferation from ducts is a background picture for transgene expression in dysplastic lesions. Nevertheless, the lack of any large T-antigen immunoreactivity in the newly formed cells did not allow us to establish a definite pathogenetic relationship between the two pathological situations. In younger animals, no lesion was observed in pituitary, suggesting late expression of the transgene in this organ as compared with the pancreas. This was confirmed indirectly by the low number of large, full blown pituitary tumours in the group of older animals. These data suggest that a tissue-specific factor showed preference for the endocrine pancreas and possibly co-operated in promoting the early expression of the transgene.

The ultrastructural labelling of large T-antigen with immunogold particles confirmed its presence in tumour cells of pancreas and pituitary and provided the opportunity to visualise its subcellular distribution. Gold particles were seen in the heterochromatin of nuclei of transformed cells. As this compartment of the nucleus is constituted by nucleoproteins and nucleic acids densely arranged, this observation gave morphological evidence that the great number of functions attributed to large T-antigen (Rigby and Lane 1983) in transformed cells are mediated by a direct interaction between large T-antigen and DNA and/or nucleoproteins.

Our finding of large T-antigen immunoreactivity in dysplastic lesions in both target organs of transgenic mice suggests that the neoplastic transformation process is initiated in mature adult cells. This contradicts the generally accepted view that endocrine tumours of the pancreas arise from multipotential ductular stem cells (Heitz 1984). In our model, the transformation process proved to be sudden and to involve well developed endocrine cells leading to the progressive loss of their hormonal synthetic properties. This was particularly evident in the pituitary where the lesions started late, leading rapidly to a high degree of cellular damage. Nevertheless, the accompanying picture of endocrine proliferation from pancreatic ducts of AVP/SV40 large T-antigen transgenic mice suggests that a proliferative pressure is a background to the transformation, as it is in the corresponding human pathology. The exact nature of the proliferative changes in AVP/SV40 transgenic mice merits further investigation.

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References

Adams TE, Alpert S, Hanahan D (1987) Non-tolerance and autoantibodies to a transgenic self antigen expressed in pancreatic β cells: Nature 324:223–227

Brinster RL, Chen HY, Messing A, Van Dyke T, Levine AJ, Palmiter RD (1984) Transgenic mice harbouring SV40 T-antigen genes develop characteristic brain tumours. Cell 37:367–379

Fiocca R, Rindi G, Capella C, Grimelius L, Polak JM, Schwartz TW, Yanaihara N, Solcia E (1987) Glucagon, glicentin, proglucagon, PYY, PP and pro-PP icosapeptide immunoreactivities of rectal carcinoid tumours and related non-tumour cells. Reg Pep 17:8–29

Hanahan D (1985) Heritable formation of pancreatic B-cell tumours in transgenic mice expressing recombinant insulin simian virus 40 oncogenes. Nature 315:115–122

Heitz PU (1984) Pancreatic endocrine tumours. In: Heitz PU, Klöppel G (eds) Pancreatic Pathology. Churchill Livingstone, Edinburgh London Melbourne New York, pp 206–232

Howell SL, Fink CJ, Lacy PE (1969) Isolation and properties of secretory granules from rat islets of Langerhans. J Cell Biol 41:154–161

Hsu SM, Rainel L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. J Histochem Cytochem 29:577–580

Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 27:137A

Klöppel G, Lewson S (1984) Anatomy and physiology of the endocrine pancreas. In: Klöppel G, Heitz PhU (eds) Pancreatic pathology. Churchill Livingstone, Edinburgh London Melbourne New York, pp 133–153

Lacy PE (1961) Electron microscopy of the Beta cell of the pancreas. Am J Med 31:851–859

Lacy PE (1962) Electron microscopy of the islets of Langerhans. Diabetes 11:509–513

Larsson LI, Ljungberg O, Sundler F, Hakanson R, Svenson SO, Rehfeld JF, Stadil F, Holst JJ (1973) Antropyloric gastrinoma associated with nesidioblastosis and proliferation of islets. Virchow Arch A (Pathol Anat) 360:305–314

Murphy D, Bishop AE, Rindi G, Murphy MN, Stamp GWH, Hanson J, Polak JM, Hogan B (1987) Mice transgenic for a vasopressin-SV40 hybrid oncogene develop tumours of the endocrine pancreas and the anterior pituitary. A possible model for human multiple endocrine neoplasia type I. Am J Pathol (in press)

Nakane PK (1970) Classification of anterior pituitary cell types with immunoenzyme histochemistry. J Histochem Cytochem 18:9–20

Orci L (1981) Banting lecture 1981 – Macro and micro domains in the endocrine pancreas. Diabetes 31:538–565

Ornitz DM, Palmiter RD, Messing A, Hammer RE, Pinkert CA, Brinster RLT (1985) Elastase I promoter directs expression of human growth hormone and SV40 T-antigen to pancreatic acinar cells in transgenicmice. Cold Spring Harbor Symp Quant Biol 50:399–409

Porte D, Halter JB (1981) The endocrine pancreas and diabetes mellitus. In: Willams RH (ed) Textbook of Endocrinology. WB Saunders, Philadelphia London Toronto, pp 716–843

Power RF, Holm R, Bishop AE, Varndell IM, Alpert S, Hanahan D, Polak JM (1987) Transgenic mouse model: a new approach for the investigation of endocrine pancreatic B-cell growth. Gut 28:121–129

Rigby PWJ, Lane DE (1983) Structure and function of Simian Virus 40 large T-antigen. In: Klein G (ed) Advance in Virol Oncology, Raven Press, New York, vol 3, pp 31–57

Scopsi L, Wang BL, Larsson LI (1986) Non-specific immunocytochemical reactions with certain neurohormonal peptides and basic peptide sequences. J Histochem Cytochem 34:1469–1476

Sternberger LA (1979) The unlabelled antibody enzyme peroxidase anti-peroxidase (PAP) method. In: Sternberger LA (ed) Immunocytochemistry. John Wiley, New York, pp 104–169

Van Noorden S (1986) Tissue preparation and immunostaining techniques for light microscopy. In: Polak JM, Van Noorden S (eds) Immunocytochemistry. Wright, Bristol, pp 26–53

Varndell IM, Tapia FJ, Probert L, Buchan AMJ, Gu J, De Mey J, Bloom SR, Polak JM (1982) Immunogold staining procedure for the localisation of regulatory peptides. Peptides 3:259–272

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