Demonstration of tissue-specific antigens shared by normal pancreas and pancreatic neoplasms

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Summary. Xenoantiserum raised against extracts of normal hamster pancreas, after absorption with normal tissues, reacted specifically with normal hamster and human pancreas by immunodiffusion. Absorbed antiserum also reacted with hamster and human pancreatic carcinoma but not with other neoplasms. Immunization of hamsters with normal pancreas extracts prevented growth of transplantable pancreatic carcinomas.

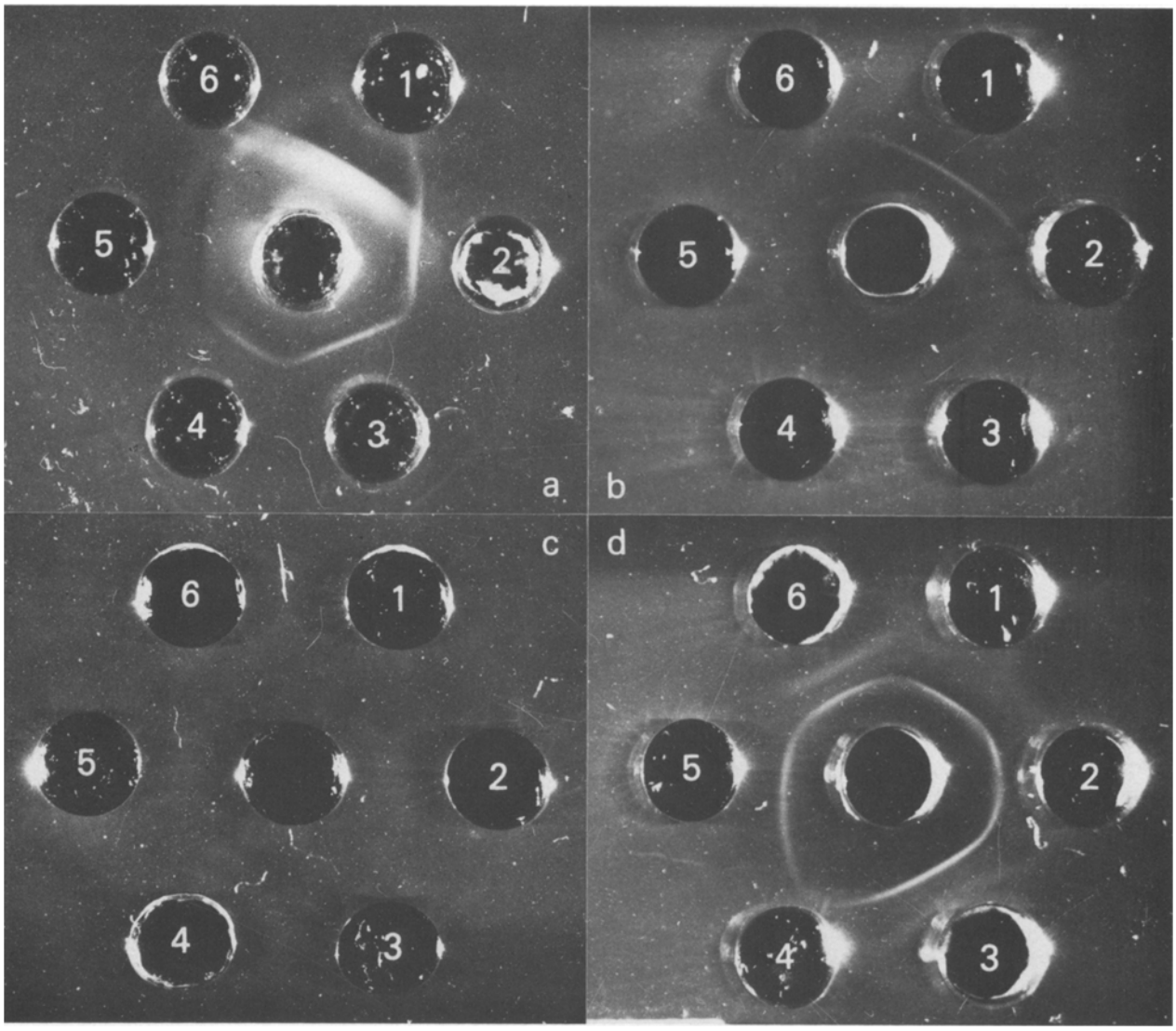
Antigens specific to normal pancreatic tissues were first reported in 1960[1]. Subsequent investigations have confirmed the existence of pancreas-specific alloantigens[2-4] and have suggested that such tissue-specific antigens are of potential importance in investigations of the immunobiology of pancreatic neoplasms. Studies of pancreatic tumors in the past have been hampered by a lack of suitable animal experimental models. However, reproducible techniques of inducing pancreatic adenocarcinomas recently have been developed in Syrian golden hamsters by the administration of nitrosamines[5-8]. Transplantable tumor lines were established, permitting experimental manipulation and investigation[9-11]. It is of considerable interest whether pancreatic neoplasms share antigens specific for normal pancreatic tissue, since the appearance of normal tissue-specific antigens on a malignant tumor could potentially serve as a means for targeting specific immunotherapy. The present study was undertaken to attempt to demonstrate tissue-specific pancreatic antigens in the hamster and to determine if normal tissue antigens were detectable in pancreatic cancers. Using immunodiffusion, it was shown that heterologous serologic reactivity exists against determinants in normal hamster pancreatic tissue. In addition, cross-reactivity exists with determinants present in hamster pancreatic cancer, human pancreatic cancer, and normal human pancreatic tissue.

Normal pancreas was harvested from 6 adult male random-bred Syrian golden hamsters, was homogenized in a 25% W/V suspension of phosphate-buffered saline, and was clarified by centrifugation at 100,000 × g for 1 h. The crude extracts contained 14 mg protein/ml and were stored at −40 °C until used for immunizations. Four male New Zealand white rabbits were immunized weekly for 6–8 weeks with the hamster pancreatic extracts. Initial immunizations were made at 4 i.m. sites with 0.1 ml of pancreatic extract emulsified in Freund's adjuvant. Subsequent immunizations were performed s.c. with 0.1 ml of pancreatic extract alone. Rabbit antiserum reactivity was tested periodically by double immunodiffusion against the original antigen, and antiserum was harvested after titers of at least 1:8 were achieved against the crude pancreatic extract. All immunized rabbits showed broad serologic reactivity in a double immunodiffusion assay against hamster serum as well as saline extracts of hamster pancreas, liver, kidney, intestine, and brain (fig. a). Hamster tissue extracts were prepared by homogenization (25% W/V) of fresh tissue in phosphate-buffered saline. Extracts were clarified by centrifugation (100,000 × g for 1 h) and were stored at −40 °C until used for testing. For each immunodiffusion test, various dilutions of the extracts were utilized ranging from 1:1 to 1:32 to be certain to achieve optimum equivalence conditions for the formation of precipitin lines. Multiple absorptions of the rabbit antisera were performed with glutaraldehyde-insolubilized hamster serum, hamster liver, and hamster kidney. Absorptions were performed with equivalent V/W for 3 h at 4 °C. Absorption of the 4 antisera resulted in the identification of a single antiserum which displayed reactivity with extracts of normal hamster pancreas but not reactivity against other hamster tissues or serum (fig. b). However, after absorption, the specific anti-pancreas antiserum remained active against normal pancreatic tissue extracts, forming precipitin lines in serum dilutions up to 1:32. Absorption of antiserum with hamster pancreas removed all detectable reactivity against hamster serum and all hamster tissue extracts tested (fig. c). Normal tissue extracts tested included hamster liver, kidney, duodenum, small intestine, colon, brain, muscle, lung, and salivary gland. The absorbed specific anti-pancreas antiserum was tested by immunodiffusion against saline extracts of a transplantable hamster pancreatic ductal adenocarcinoma (CBP)[11], of normal human pancreas obtained at autopsy (3 patients), and of human pancreatic adenocarcinoma obtained at surgery (3 patients). Prior to testing against human tissues, the antiserum was absorbed with glutaraldehyde-insolubilized pooled human serum. Tissue extracts were prepared by homogenization (25% W/V) in phosphate-buffered saline and by centrifugation to clarity (100,000 × g for 1 h). Precipitin lines of identity were formed by the absorbed anti-pancreas antiserum reacting against normal hamster pancreas, normal human pancreas, hamster pancreatic carcinoma, and human pancreatic carcinoma (fig. d). The degree of reactivity varied against the human pancreatic tissues and the human pancreatic cancers, and the reactivity against human tissues was consistently less than that against hamster pancreas or hamster pancreatic tumor. However, cross-reacting antigens were present in all extracts tested. Precipitin lines appeared in addition to the major anti-hamster normal pancreas band when extracts of human pancreatic cancers were reacted, but such lines were weak in intensity. The additional bands may have represented natural xenogeneic antibodies in the rabbit antisera. No bands in addition to the major anti-hamster normal pancreas band appeared in immunodiffusion plates reacting absorbed anti-hamster pancreas serum against extracts of normal human pancreas. No significant reactivity was seen of absorbed anti-hamster pancreas serum against extracts of normal human liver, kidney, small intestine, colon, or muscle, or against extracts of human gastric carcinoma (1 patient), colon carcinoma (2 patients), or soft tissue sarcoma (4 patients).

Absorbed anti-hamster pancreas serum was used in indirect immunoperoxidase staining of normal hamster pancreas and CBP hamster pancreatic carcinoma. Tissues were fixed in formalin, dehydrated, embedded in paraffin, and sectioned. Sections were stained with dilutions up to 1:20 of the rabbit anti-hamster pancreas serum for 1 h as the primary antibody and with peroxidase-conjugated goat anti-rabbit antiserum at 1:40 dilution for 1 h as the secondary antibody. Sections of normal hamster pancreas showed peroxidase staining in the apical portions of the epithelial cells forming the pancreatic ducts and ductules. Staining was typically uniform, with > 85% of cells showing the presence of reaction product. Sections of the CBP hamster pancreatic carcinoma showed deposition of stain in malignant cells which formed ductal patterns. Staining was

present in approximately 55% of tumor cells, with a considerable variation in the staining intensity among cells.

The results indicate that antigenic determinants exist in normal pancreatic tissue in the hamster which also are expressed in the CBP hamster pancreatic carcinoma. Furthermore, cross-reacting determinants are also expressed in both human normal pancreatic tissue and human pancreatic carcinomas. The character and significance of the pancreatic tissue-specific antigens are unknown at present and are currently being investigated. It is possible that pancreatic-specific antigens could be exploited as a means of targeting immunotherapy of pancreatic neoplasms, if the antigens are present on neoplastic as well as normal tissues. In the hamster model system, reaction to pancreatic-specific determinants does seem to have the potential for providing immunity to the CBP transplanted pancreatic carcinoma. Ten inbred CB strain male hamsters were inoculated i.m. with 0.1 ml of a homogenate of normal hamster pancreas emulsified in complete Freund's adjuvant (equal V/V) in an attempt to hyperimmunize against pancreas-associated antigens. Animals were inoculated weekly for 4 weeks. Ten control animals received Freund's alone. An additional 10 animals had surgical excision of CBP pancreatic carcinoma (tumors measuring approximately $5 \times 5 \times 5$ mm) growing s.c. in the scruff; the tumors had been transplanted by inoculation of 1×10^6 CBP cells 7 days previously. All animals were inoculated with a challenge dose of 1×10^6 CBP cells, a dose which produces 100% tumor incidence in normal inbred CB hamsters. All animals were observed for tumor growth at the challenge site.



Immunodiffusion plates of rabbit antiserum raised against normal hamster pancreatic tissue tested against extracts of various hamster and human tissues. *a* Crude anti-pancreas serum in center well showing broad specificities against hamster tissues. Well 1 contains hamster pancreas extract; 2, liver; 3, intestine; 4, kidney; 5, hamster serum; 6, brain. *b* Anti-pancreas serum in center well after absorption with insolubilized hamster serum, liver, and kidney. Single specificity is present against normal hamster pancreas. Well 1, pancreas; 2, liver; 3, intestine; 4, kidney; 5, hamster serum; 6, brain. *c* Anti-pancreas serum in center well after absorption with hamster pancreas. All reactivity is removed against hamster tissues. Well 1, pancreas; 2, liver; 3, intestine; 4, kidney; 5, hamster serum; 6, brain. *d* Anti-pancreas serum in center well after absorption with hamster serum, hamster liver, hamster kidney, and human serum. Precipitin line of identity is seen between hamster pancreas, CBP hamster pancreatic carcinoma, normal human pancreas, and human pancreatic carcinomas. Well 1, normal hamster pancreas; 2, CBP hamster pancreatic carcinoma; 3, normal human pancreas; 4, normal human liver; 5, human pancreatic carcinoma (well-differentiated); 6, human pancreatic carcinoma (poorly-differentiated).

All 10 control animals developed tumors. Two of 10 animals having excision of previously-inoculated CBP tumors developed tumors after subsequent challenge ($p < 0.01$ by χ^2 analysis as compared to controls). Four of 10 hamsters immunized with normal pancreas developed tumors after challenge ($p < 0.01$), indicating that a level of tumor immunity was induced presumably through determinants in the normal pancreatic tissue. Further studies are necessary to characterize pancreatic-specific antigens and to determine whether similar classes of such antigens exist in various species. Examination of various pancreatic neoplasms is necessary before substantial inferences can be made as to whether the expression of normal tissue antigens is a characteristic of pancreatic cancers.

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ABO system incompatibility: evaluation of risk of hyperbilirubinaemia at birth by multivariate discriminant analysis[1]

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Summary. A discriminant analysis was performed on a set of maternal and neonatal variables to predict at birth the serum bilirubin levels during the neonatal period in infants incompatible with their mothers in the ABO system. The results suggest that the rational and simultaneous utilization of clinical and laboratory parameters allows, a few hours after delivery, a useful classification of these infants in low or high risk for hyperbilirubinemia.

ABO feto-maternal incompatibility shows a high prevalence both in Causasian and in Negro populations. Although severe ABO hemolytic disease is rare, milder forms are relatively frequent: in these cases jaundice may not be detected soon after birth and early discharge of the newborn may have serious consequences[3]. Therefore, in order to select infants which may be discharged in the very first days of life, the early identification of the newborns at risk of hyperbilirubinaemia is very important.

In the present paper we report a discriminant analysis performed on a set of maternal and neonatal variables to predict at birth the serum bilirubin levels during the neonatal period.

The analysis was performed according to Klecka[4] on a IBM 370/158 computer. By this procedure a linear combination of independent variables (discriminant function) that best distinguish between cases in the categories of the dependent variable (bilirubin level) is found. The most useful variables can be selected by stepwise procedure. Variables which are not able to contribute to discrimination according to a user-determined criterion (a fixed value of multivariate F ratio) are not included in the discriminant function. Several indexes of discriminating power of single variables and of the importance of discriminant functions are provided by SPSS Discriminant subprogram.

302 White newborns of European descent and 76 Black infants incompatible with their mother only in the ABO system were studied. The sample was collected at the Yale New Haven Hospital. Biochemical, immuno-hematological and sampling methods were reported in previous papers[5-8].

Table 1. Variables used for the discriminant analysis

Variable included in the final analysis	Categories
Gestational age	
Birth weight	
Birth order	First or second
	Third or higher
ABO maternal phenotype	A or B
	O
Type of feto-maternal	A
ABO incompatibility*	B
Direct coombs test	Negative
	Positive
Presence of $P1^{f1}$ allele of placental	Both present
alkaline phosphatase** and of I^B	Only one or none present
allele of ABO system	
Dose of $P1^{i1}$ or rare alleles of	Absent
placental alkaline phosphatase**	Heterozygous
	Homozygous

*Used only in Blacks. **Placental alkaline phosphatase (PAP) is a polymorphic enzyme which is produced by the fetus and is found in the maternal circulation during gestation. This polymorphic system is controlled by an autosomal locus with 3 common alleles ($P1^{s1}$, $P1^{f1}$ and $P1^{i1}$) and a high number of rare alleles. We had previously observed, in ABO incompatible newborn infants, a positive association between the direct Coombs test and the incidence of jaundice and a negative association between the latter and the simultaneous presence of I^B and $P1^{f1}$ factors[5,6]. Variables discarded. Maternal age. Previous spontaneous abortions. PGM_1 phenotype. Dose of $P1^{f1}$ factor of placental alkaline phosphatase. Enzymatic activity of placental alkaline phosphatase phenotype.