kidney transplantation between HL-A identical unrelated individuals and the difficulty of establishing a statistically significant correlation between HL-A matching grade and clinical outcome of the graft (Stewart *et al.*, 1970) indicate that the observations in siblings cannot be directly transposed to the unrelated situation. If the HL-A locus, as currently defined by two segregant series, is not solely responsible for mixed lymphocyte culture reactivity the additional factors responsible for this reactivity may also contribute to histocompatibility and be relevant to transplantation. Elucidation of these factors could help us to define histoidentity more precisely and then the results of transplantation with unrelated cadaveric donors may more closely approach the excellent results of HL-A identical sibling transplants.

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Transience of Immune Responses to Tumour Antigens in Man

J. L. ODILI, GEOFFREY TAYLOR

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Summary

By examining serial serum samples from patients undergoing surgical removal of malignant tumours an increased incidence of tumour-specific antibodies has been detected. Antibody responses to tumour neoantigens seem to be transient, and this is held to account for the rarity of detections where a single sample of serum is examined.

Introduction

A large body of opinion holds that human tumours, like experimental animal tumours, possess tumour-specific antigens (tumour neoantigens) (Fairley, 1969, 1970; Mathé, 1969, 1970; Alexander, 1970; Klein, 1970; Woodruff, 1970). Yet only in a minority of cancer patients have antibody responses to tumour neoantigens been detected (Graham and Graham, 1955; Wilson *et al.*, 1963; Southam, 1965; Dore *et al.*, 1967; Lewis, 1967; Lewis *et al.*, 1969).

In a previous communication from this department, Hodkinson and Taylor (1969) reported the detection of tumour-specific antibodies in the sera of only 2 out of 34 cases of human carcinoma. One reason suggested for the low frequency of tumour antibody detection was the possibility that while the tumour is in situ a slow constant release of tumour antigen combines with any antibody produced so that the latter is not detectable in the serum. The work reported here is an investigation of this possibility, and the findings suggest that reaction in vivo between tumour neoantigens and tumour antibodies could be a reason for the low frequency of antibody detection.

Materials and Methods

Tumour and corresponding normal tissues from the same individual were obtained from surgical specimens within half an hour of excision. Venous blood samples were obtained from each patient providing the tissues as follows: sample 1, obtained preoperatively on the day of operation or on the previous day; sample 2, obtained three to six days postoperatively; sample 3, obtained 10 to 13 days postoperatively; and sample 4, obtained when the patient attended the follow-up clinic, two to four months postoperatively.

Four cellular fractions (nuclear, mitochondrial, microsomal, and supernatant) of both normal and carcinomatous tissue were tested by immunofluorescence, complement fixation, passive haemagglutination, and precipitation techniques against autologous sera in attempts to detect tumour-specific antibodies. The preparation of cellular fractions from tumour and normal tissue specimens, the preparation of serum, and the serological techniques used have been fully described elsewhere (Hodkinson and Taylor, 1969; Taylor and Odili, 1970). Tissue specimens, sera, and tissue fractions were stored at —25°C until required. Tissues and sera from 36 patients were studied.

Results

Tumour-specific antibody responses were detected in 5 (14%) of the 36 cancer patients studied (Table I). In four of the five positive cases the autologous sera reacted against the nuclear fraction of the tumours; in the fifth case the serum reactivity was directed against the microsomal fraction. Complement fixation was by far the best method of antibody detection. The positive sera in all five cases gave negative tests against the corresponding autologous normal tissue fractions—that is,

TABLE I—*Tumour-specific Antibody Detection in Sera of Cancer Patients and its Relation to Tumour Types*

Tumour Type	Number Studied	Antibody Detected	
		Number	% of Total No. of Cases
Carcinoma of breast	11	1	2·8
„ „ oesophagus ..	2	0	0
„ „ stomach ..	6	2	5·6
„ „ colon	2	1	2·8
„ „ rectum	8	1	2·8
„ „ lung	4	0	0
„ „ kidney	1	0	0
„ „ ovary	1	0	0
„ „ scrotum ..	1	0	0
Total	36	5	14

Immunology Department, Royal Infirmary, Manchester 13

J. L. ODILI, M.B., B.S., PH.D., Research Registrar
GEOFFREY TAYLOR, M.D., D.PATH., Reader in Immunology; Consultant Immunologist

the antibodies were strictly tumour specific. All sera giving positive results were retested at least three times against both tumour and corresponding normal tissue fractions and consistent results were obtained. Table II presents the detailed results of the positive cases. The two positive sera directed against intestinal tract cancer neoantigen (colon and rectum, Cases 2 and 4) failed to react with purified carcinoembryonic antigen derived from both fetal ileum and carcinoma of colon (Gold and Freedman, 1965). The two positive sera from patients with carcinoma of the stomach (Cases 3 and 5) did not react by fluorescent antibody technique with several samples of normal human gastric mucosa, and the positive serum from the patient with breast cancer (Case 1) failed to react with extracts of normal breast (10) or with extracts of carcinoma of stomach (3), carcinoma of rectum (5), carcinoma of colon (1), or carcinoma of oesophagus (1).

Table III is a summary of the positive results and shows the time relationship of tumour antibody detection to operative excision of tumour. In Cases 1 and 2 tumour antibody was detected in the preoperative serum samples, and there was a rise in antibody titre, as indicated by the units of complement fixed, in the postoperative serum samples. In Cases 3 and 4 antibody was not detected in the preoperative serum samples but was detected in the samples taken in the "immediate" postoperative period. Case 5 had demonstrable antibody in the preoperative serum but the antibody was not detectable postoperatively. One factor common to all five cases was the transient nature of the antibody responses. Antibody was not detected in the serum samples obtained some considerable time after operation, with the exception of Case 1 in whom the peak antibody titre was detected on the 56th postoperative day.

TABLE II—*Fixation of Complement with Sera and the Nuclear (Cases 1, 2, 3, and 5) and Microsomal (Case 4) Fractions*

Serum Obtained	Serum Dilutions	Antigen Dilutions 1/4	1/10	1/25
Case 1: Carcinoma of Breast				
1 day preop.	1/5	1·52*	1·38	0·9
	1/25	0·60	0	0
	1/125	0	0	0
Day 4 postop.	1/5	3·0	2·44	1·12
	1/25	1·0	0·72	0·60
	1/125	0	0	0
Day 10 postop.	1/5	3·20	2·96	1·56
	1/25	1·25	0·85	0·60
	1/125	0	0	0
Day 56 postop.	1/5	4·50	4·31	1·88
	1/25	2·85	2·2	0·75
	1/125	0	0	0
Day 214 postop.	1/5	1·34	0·95	0
	1/25	0	0	0
	1/125	0	0	0
Case 2: Carcinoma of Colon				
Preop. day of op.	1/5	1·20	0	0
	1/25	0	0	0
	1/125	0	0	0
Day 5 postop.	1/5	1·47	0	0
	1/25	0	0	0
	1/125	0	0	0
Day 12 postop.	1/5	2·12	0	0
	1/25	0	0	0
	1/125	0	0	0
Day 83 postop.	1/5			
	1/25	0	0	0
	1/125			
Case 3: Carcinoma of Stomach				
Preop. day of op.	1/2			
	1/5	0	0	0
	1/25			
	1/125			
Day 4 postop.	1/2	2·45	1·62	0
	1/5	1·25	1·00	0
	1/25	0	0	0
	1/125	0	0	0
Day 13 postop.	1/2	2·50	2·12	0
	1/5	1·87	1·00	0
	1/25	0	0	0
	1/125	0	0	0
Day 110 postop.	1/2			
	1/5	0	0	0
	1/25			
	1/125			
Case 4: Carcinoma of Rectum				
Day 1 preop.	1/5			
	1/25	0	0	0
	1/125			
Day 4 postop.	1/5	1·1	0	0
	1/25	0	0	0
	1/125	0	0	0
Day 10 postop.	1/5	1·25	0	0
	1/25	0	0	0
	1/125	0	0	0
Day 54 postop.	1/5			
	1/25	0	0	0
	1/125			
Case 5: Carcinoma of Stomach				
Preop. day of op.	1/5	2·66	2·16	1·12
	1/25	1·56	1·52	0
	1/125	0	0	0
Day 5 postop.	1/5			
	1/25	0	0	0
	1/125			
Day 12 postop.	1/5			
	1/25	0	0	0
	1/125			
Day 109 postop.	1/5			
	1/25	0	0	0
	1/125			

* The numbers represent the units of complement fixed. Fixation of 0·5 or less was not regarded as significant.

TABLE III—*Relationship of Tumour Antibody Detection to Surgical Removal of Tumour*

Case No.	Preoperative Serum 1	Postoperative Serum Samples Serum 2	Serum 3	Serum 4	Serum 5
1	+	++	++	+++	+
2	+	+	++	−	
3	−	++	++	−	
4	−	+	+	−	
5	++	−	−	−	

+ and − indicate presence or absence of detectable antibody; the pluses also indicate the degree of antibody response.

Discussion

By searching for tumour antibody responses in cancer patients, both before and at intervals after surgical removal of their tumours, the incidence of antibody detection by the techniques used in this laboratory has been increased from 6% (Hodkinson and Taylor, 1969) to 14% reported here. Though the frequency of antibody detection is still relatively low, two points have emerged: (1) four of the five positive cases showed a rise in antibody titre after removal of their tumours—two of these four had weak or undetectable circulating antibody while their tumours were in situ; and (2) all the observed antibody responses were transient in nature, varying in degree and duration in all five cases (Tables II and III).

The rise in titre of tumour antibody and its apparent appearance in the circulation after tumour removal suggest that while the tumour is in situ any antibody formed against it is constantly being absorbed by the tumour. The transient nature of the antibody response indicates that tumour antibody responses could be commoner than is realized but because of their timing may be completely missed. Weak reactions detected at one time, and which are not followed to their peak, are ignored and classed as negative. By testing serial serum samples we have also detected transient antibody responses in 2 out of 12 patients with advanced malignant disease undergoing active immunotherapy (to be published). Graham and Graham (1955) detected tumour autoantibodies in 12·5% of 48 cancer patients before surgical treatment. After tumour removal antibody was detected in a further 12·5%.

Another possible reason for the rise in titre of tumour antibody and its improved detection after tumour removal is the fact that manipulation of tumours at operation may lead to release of live tumour cells into the circulation, and the neoantigens of the released tumour cells could act as further stimulus for antibody production. Since most malignant tumours undergo some degree of necrosis, implying that intracellular antigens are released, it may be that neoantigens from necrotic tumour cells are not as immunogenic as those of live or whole tumour cells. The demonstration by Ikonopisov *et al.* (1970), that of 13 patients with malignant melanoma in whom antibodies were not detectable the subcutaneous injection of irradiated autologous tumour cells resulted in the development of tumour antibodies in nine, may be supportive evidence for this suggestion. In the cases reported by Ikonopisov *et al.* (1970) the antibody response was also transient, varying from six days in patients with widespread disease to 10 to 14 days in those whose tumour spread was limited to the regional lymph nodes.

No significant difference has been observed in the course of the disease between the patients who had detectable antibodies and the antibody-negative ones. Two of the patients who had demonstrable tumour antibody died 6 and 12 months after operation respectively. The other three are still alive at the time of writing and have survived 29, 21, and 15 months since operation. Of the 31 antibody-negative patients, 14 have died, their postoperative survival time ranging from 1 to 25 months (mean 9·8 months). The remaining 17 antibody-negative patients, who are still alive, have a postoperative survival time ranging from 11 to 29 months (mean 19 months).

Thus while it cannot be said that antibody responses in the five positive cases have altered the course of the disease for the better, there has been no evidence suggesting that the antibodies detected enhanced the growth of any residual tumour in these patients. From the pattern of antibody responses detected in this study we conclude that in vivo reaction between tumour neoantigens and tumour antibodies could be a reason for the low frequency of detection of circulating tumour antibodies in man.

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Clinical Evaluation of Perhexiline Maleate in Patients with Angina Pectoris

C. J. BURNS-COX, K. P. CHANDRASEKHAR, H. IKRAM, T. H. PEIRCE, J. PILCHER, C. D. M. QUINLAN, J. RUSSELL REES

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Summary

This paper reports a double-blind trial of a new antianginal drug, perhexiline. Fifty-five patients suffering from angina pectoris were studied for periods of 12 or 24 weeks in a cross-over comparison against a placebo in four centres in the United Kingdom and Ireland. Perhexiline was effective in most patients as judged by reducing the number of anginal attacks in 84% and the consumption of glyceryl trinitrate tablets in 64%. The major side effect, dizziness, noted in one-third of the patients, may be dose/body-weight related. Perhexiline is a valuable new agent for the treatment of patients with angina, especially those who do not respond to other antianginal agents.

Bristol General Hospital, Bristol BS1 6SY
C. J. BURNS-COX, M.B., B.S., M.R.C.P., Senior Medical Registrar
J. RUSSELL REES, M.D., M.R.C.P., Consultant Physician

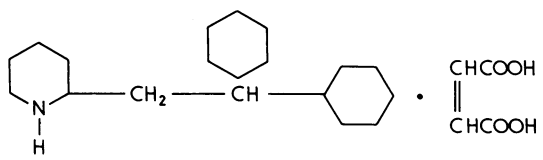
United Sheffield Hospitals and Sheffield Regional Hospital Board
K. P. CHANDRASEKHAR, M.D., M.R.C.P.ED., Senior Medical Registrar
J. PILCHER, M.B., B.CHIR., M.R.C.P., Senior Medical Registrar

Charing Cross Hospital, London WC2N 4DZ
H. IKRAM, M.B., CH.B., M.R.C.P., Senior Medical Registrar

Adelaide Hospital, Dublin C.8
T. H. PEIRCE, M.B., B.CH., B.A.O., Medical Registrar
C. D. M. QUINLAN, M.B., B.CH., M.R.C.P., Senior Medical Registrar

Introduction

Perhexiline maleate is a new antianginal drug which is related to hexadylamine, a known coronary vasodilator. Its chemical formula is:



Perhexiline's actions in animals have been described by Hudak *et al.* (1970) and by Cho *et al.* (1970). These are: (1) systemic and coronary arterial vasodilation, (2) increase in coronary arterial and venous blood flow, (3) slowing of the heart rate, and (4) increase in pulmonary compliance.

Studies with perhexiline in man have been done in the United States and Brazil (Hirshleifer, 1969; Martins de Oliveira