sometimes yield false results especially during early pregnancy (*13*). The radioreceptor assay reported here is rapid, specific, and sensitive enough to detect pregnancy as early as day 6 to 8 after conception, and is of use in the diagnosis and clinical management of early pregnancy, abortion, ectopic pregnancy, infertility, related pathophysiological conditions, and in the evaluation of suction curettage and artificial insemination (*14*).

B. B. SAXENA, S. H. HASAN
F. HAOUR, M. SCHMIDT-GOLLWITZER
Division of Endocrinology,
Department of Medicine,
Cornell University Medical College,
New York 10021

References and Notes

1. J. Roth, *Metabolism* **22**, 1059 (1973).
2. S. G. Korenman, D. Tulchinsky, L. W. Eaton, in *Steroid Assay by Protein Binding*, E. Diczfalusy, Ed. (Karolinska Institute, Stockholm, 1970), p. 291; R. Lefkowitz, J. Roth, I. Pastan, *Science* **170**, 633 (1970); T. Tsuruhara, E. V. Van Hall, M. L. Dufau, K. J. Catt, *Endocrinology*, **91**, 463 (1972); T. Tsushima, and H. G. Friesen, *J. Clin. Endocrinol. Metab.* **37**, 334 (1973); A. R. Means and J. Vaitukaitis, *Endocrinology* **90**, 39 (1972).
3. The abbreviations used in this text are: hCG, human chorionic gonadotropin; LH, luteinizing hormone; hGH, human growth hormone; FSH, follicle stimulating hormone; ACTH, adrenocorticotropic hormone; TSH, thyroid stimulating hormone; PRL, prolactin.
4. D. Gospodarowicz, *J. Biol. Chem.* **248**, 5050 (1973).
5. F. Haour and B. B. Saxena, *ibid.* **249**, 2195 (1974).
6. ———, in preparation; F. M. Finn, C. C. Widnell, K. Hoffman, *J. Biol. Chem.* **247**, 5695 (1972).
7. The hCG was provided by Dr. O. P. Bahl and Dr. R. E. Canfield.
8. J. T. Thorell and B. G. Johansson, *Excerpta Med. Int. Congr. Ser.* **241**, 531 (1972).
9. B. B. Saxena, P. Rathnam, A. Rommler, *Endocrinol. Exp.* **7**, 19 (1973).
10. F. Haour and B. B. Saxena, *Science*, in press.
11. H. F. Klinefelter, Jr., F. Albright, G. C. Griswold, *J. Clin. Endocrinol.* **3**, 529 (1943).
12. D. P. Goldstein, J. Miyata, M. L. Taymor, L. Levesque, *Fertil. Steril.* **23**, 817 (1972); L. Wide and J. Porath, *Biochim. Biophys. Acta* **130**, 256 (1967); G. D. Braunstein, J. M. Grodin, J. Vaitukaitis, G. T. Ross, *Am. J. Obstet. Gynecol.* **115**, 447 (1973).
13. S. Brody and G. Carlstorm, *J. Clin. Endocrinol.* **22**, 564 (1962).
14. We thank N. Moore for technical assistance. Supported by NIH contract NIH-NICHD-2-2763 and NIH grants CA-13908 and HD-06543, and grant 670-0455A from The Ford Foundation. Part of this work has been presented in the proceedings of the International Round Table Conference on "Hormones in Pregnancy" at Fresnes, France in October 1973. B.B.S. is a career scientist awardee, Health Research Council of the City of New York, contract I-621. S.H.H., Schering, A.G., Germany. The committee on terminology of the American College of Obstetrics and Gynecology [*Obstetrical-Gynecologic Terminology*, E. C. Hughes, Ed. (Davis, Philadelphia, Pa., 1972), pp. 299 and 372] states "pregnancy is the state of a female after conception and until termination of the gestation" whereas "conception is the implantation of the blastocyst." Hence, Dr. Philip A. Corfman, M.D., Director, Center for Population Research, National Institute of Child Health and Human Development, Bethesda, Md. 20014, has suggested that a test that detects "pregnancy" prior to implantation should be called a "fertilization test."

2 October 1973; revised 28 December 1973

Serum Induced Lymphoid Cell Mediated Cytotoxicity to Human Transitional Cell Carcinomas of the Genitourinary Tract

Abstract. *In some serums of patients with transitional cell carcinoma (TCC), a factor is present which induces lymphocytes from most donors with or without TCC to become cytotoxic against TCC-derived target cells. The induced cytotoxicity was directed against target cells derived from TCC's of the renal pelvis, ureter, and urinary bladder, but not against cells derived from normal kidney, bladder, testis, or skin or from renal cell carcinoma. Cytotoxicity occurred without complement but did not occur without effector cells.*

In animals, tumor-specific, lymphocyte mediated cytotoxicity (LMC) has been demonstrated by means of assays in vitro (*1*). This in vitro LMC can be abrogated by the addition of serum factors present in so-called blocking serums (*1*). In contrast to the reduction in LMC caused by blocking serums, it has been demonstrated that certain immune serums contain other factors (lymphocyte dependent antibodies) which induce tumor-specific cytotoxicity by lymphocytes from immune and nonimmune donors (*2*). This lymphocyte dependent antibody (LDA) is an immunoglobulin G (IgG) which does not

Table 1. Target cell survival after exposure to LDA-like activity of serums from patients T5 and T6 with and without effector cells (lymphocytes). Serum donors T5 and T6 were diagnosed as having transitional cell carcinoma of the bladder; donors C1 and C3 were normal. All serums were diluted 1 : 5 in phosphate buffered saline, except as indicated; S.E.M., standard error of mean; CI, cytotoxic index; ns, not significant.

Lymphocyte donor (diagnosis)	Serum donor	Surviving cells			
		Type	Number ± S.E.M.	CI	*P*
		Experiment 1			
None	C3	TCC-bladder	78.0 ± 9.0		
None	T6		93.5 ± 9.3	−20	ns
T1 (TCC-pelvis)	C3		76.2 ± 8.5		
T1	T6		15.0 ± 1.8	80	.01
None	C3(1 : 20)		74.3 ± 9.4		
None	T6(1 : 20)		68.3 ± 8.6	8	ns
T1	C3(1 : 20)		79.5 ± 2.1		
T1	T6(1 : 20)		49.2 ± 6.6	38	.01
		Experiment 2			
None	C3	TCC-pelvis	52.2 ± 1.6		
None	T5		46.7 ± 3.0	11	ns
T1 (TCC-pelvis)	C3		28.2 ± 4.4		
T1	T5		13.2 ± 1.1	53	.01
C1 (normal)	C3		29.3 ± 1.5		
C1	T5		28.0 ± 1.6	5	ns
T2 (TCC-bladder)	C3		17.2 ± 1.5		
T2	T5		10.2 ± 1.0	41	.01
T3 (TCC-bladder)	C3		35.2 ± 1.9		
T3	T5		29.0 ± 1.8	18	.05
		Experiment 3			
None	C1	TCC-bladder	52.5 ± 3.6		
None	T5		64.8 ± 19.0	−23	ns
T1 (TCC-pelvis)	C1		18.0 ± 1.8		
T1	T5		0.8 ± 0.2	96	.01
T2 (TCC-bladder)	C1		51.4 ± 5.4		
T2	T5		0.5 ± 0.3	99	.01
T4 (TCC-bladder)	C1		36.5 ± 6.7		
T4	T5		1.0 ± 0.6	97	.01
C2 (normal)	C1		63.8 ± 7.5		
C2	T5		15.6 ± 2.3	76	.01
None	C1	Normal kidney	73.3 ± 8.8	− 5	ns
None	T5		77.0 ± 3.0		
T1 (TCC-pelvis)	C1		44.6 ± 7.0	− 4	ns
T1	T5		46.4 ± 4.2		
T2 (TCC-bladder)	C1		57.0 ± 6.0	2	ns
T2	T5		56.0 ± 4.8		
T4 (TCC-bladder)	C1		52.0 ± 3.6	12	ns
T4	T5		45.8 ± 2.7		
C2 (normal)	C1		47.3 ± 2.4	− 3	ns
C2	T5		48.7 ± 2.6		

Table 2. Summary of lymphocyte cytotoxicity response to serum LDA activity against TCC and control target cells. The diagnoses of the lymphocyte donors were: TCC-P, TCC of the renal pelvis; TCC-B, TCC of the bladder; RCC, renal cell carcinoma; BPH, benign prostatic hyperplasia. Control target cells were derived from the following tissues: RCC, renal cell carcinoma; NB, normal bladder; NK_A, NK_B, NK_C, normal kidney from donors A, B and C; NT, normal testis; NS, normal skin. +, significant lymphocyte cytotoxicity induction against indicated target cell; −, no significant cytotoxicity induction; in no experiment did serums with LDA-like activity cause significant cytotoxicity against any target cell when tested without effector cells (lymphocytes). Multiple entries under a given target cell indicate results of replicate testing of lymphocytes from the same donor on different dates. Mean induced lymphocyte cytotoxicity among + donors was equal to 83.6.

Lymphocyte donor	Target cell origin									
	TCC-bladder	TCC-pelvis	TCC-ureter	RCC	Normal					
					NB	NK_A	NK_B	NK_C	NT	NS
T1 (TCC-P)	+,+,+,+	+				−	−	−		
T2 (TCC-B)	+,+,+	+					−	−	−	−
T3 (TCC-B)	+	+						−		
T4 (TCC-B)	+,+,+	+	+	−	−		−	−		
T7 (TCC-B)	+,+		+							
T8 (TCC-B)	+			−						
T9 (TCC-B)	+						−			
T10 (TCC-B)	+					−				
T11 (TCC-B)	+,+					−				
T12 (TCC-B)	+									
T13 (TCC-B)	+									
C1 (Normal)	+,+	−				−				
C2 (Normal)	+									
C3 (Normal)	−				−	−				
C4 (RCC)			+	−						
C5 (RCC)	+			−						
C6 (Normal)	+						−			
C7 (BPH)	+			−						
C8 (Normal)	+,+					−				
C9 (Stricture)	−									

require complement for its activity and which acts in concert with thymus independent lymphocytes bearing receptors for the Fc portion of IgG (*3*).

In man, the existence of tumor-specific LMC and serum "blocking" activity have been reported in a variety of tumors (*1*), including transitional cell carcinomas (TCC) of the genitourinary tract (*4*). However, tumor-specific LDA activity has not been described in association with human solid tumors. We now report that a factor having LDA-like activity directed against the solid tumor TCC exists in a small percentage of TCC patient serums (2/40 TCC serums, 0/42 non-TCC serums), and that this factor can induce lymphocyte cytoxicity directed preferentially against TCC-derived target cells.

We measured serum LDA-like activity by a modification of the microcytotoxicity test described by Hellstrom *et al.* (*5*). Target cells were derived from TCC's arising in the renal pelvis (TCC-pelvis), ureter (TCC-ureter), and urinary bladder (TCC-bladder), as well as cells from normal kidney, bladder, testicle, skin, and renal cell carcinoma, and were prepared as described (*6*). The cell line T24 derived from a human TCC-bladder (*7*) was provided by Dr. Jorgen Fogh (Sloan-Kettering Institute, New York). Target cells were propagated as monolayer cultures in Waymouth medium supplemented with 10 percent tryptose phosphate broth (Difco), 20 percent fetal calf serum, penicillin (100 I.U./ml), streptomycin (100 μg/ml), and glutamine (1 m*M*) and were grown in a humidified atmosphere of 5 percent carbon dioxide in air at 37°C. All target cells were without evidence of mycoplasma by culture and electron microscopic examination.

Mononuclear effector cells (lymphocytes) from defibrinated venous blood samples from 11 TCC patients and 9 control patients without TCC were obtained by density gradient centrifugation in Ficoll-Hypaque (*8*), or by plasma-gel sedimentation (*9*) and incubation on nylon fibers (*10*) with subsequent red cell lysis in ammonium chloride (*11*). The mononuclear effector cell preparations studied contained more than 95 percent lymphocytes. Serum samples were obtained from 40 TCC patients and from 42 control patients with non-TCC cancer or without malignancy. The complement activity of the serums was destroyed by heating at 56°C for 30 minutes, and the serums were stored at − 120°C in the vapor phase of liquid nitrogen.

The microcytotoxicity assay was performed as follows. Target cells (averaging 50 to 100 target cells per well) were added to each well of a Micro-Test II plate (No. 3040, Falcon Plastics) and incubated overnight to allow attachment of the cells. The growth medium was then decanted, and 0.1 ml of serum, diluted 1 : 5 in 0.01*M* phosphate-buffered saline (PBS), *p*H 7.4, was added to wells containing the target cells. The target cells were incubated for 60 minutes on a rocking platform at 37°C, and then the lymphocytes (2×10^5 cells in 0.1 ml of medium without fetal calf serum) were added to each well. After further incubation for 1 hour, 0.05 ml of growth medium containing 50 percent fetal calf serum (the complement was inactivated by heating) was added to each well and incubation was resumed for 48 hours more to allow maximum expression of cytotoxicity. After this incubation, the media and unattached cells were removed by gentle washing with PBS. The surviving, attached target cells were fixed with absolute ethanol, stained with crystal violet (0.1 percent), visualized under a microscope, and counted. Cytotoxic effects were estimated by comparing the number of target cells remaining after exposure to lymphocytes from a given donor plus test serum with the number of target cells surviving exposure to lymphocytes from the same lymphocyte donor plus control serum. Target cell reduction was expressed as a cytotoxic index (CI) derived as follows:

$$\frac{N_{CS} - N_{TS}}{N_{CS}} \times 100 = CI$$

where N_{CS} is the number of target cells surviving exposure to lymphocytes plus control serum, and N_{TS} is the number of target cells surviving exposure to lymphocytes plus test serum. Cytotoxicity was considered significant if the difference between the number of target cells surviving exposure to test and to control reagents had a *P* value of less than .05 by Student's *t*-test.

Table 1 shows the LDA-like activity of serum from TCC-bladder patient T6 in inducing cytotoxicity in lymphocytes against TCC-bladder–derived target cells (Table 1, experiment 1). Lymphocytes from TCC donor T1 significantly reduced TCC-bladder target cell survival in the presence of serum from patient T6 as compared to target cell survival after exposure to lymphocytes from the same donor (T1) plus serum

from the control patient C3. These differences in target cell survival were not the result of a nonspecific growth promoting effect by the control serum (C3) because a similar number of target cells remained after exposure to either T6 or C3 serum in the absence of lymphocytes. The induction of lymphocyte cytotoxicity by the T6 serum was dependent on concentration because further dilution of T6 serum (1/20) resulted in a diminution of the induced lymphocyte cytotoxicity against the TCC-bladder target cell (Table 1, experiment 1). Lymphocytes from most, but not all, lymphocyte donors were rendered cytotoxic to TCC-derived target cells by the LDA-like serum activity (Table 1, experiments 2 and 3). While lymphocyte cytotoxicity was induced against the TCC-bladder target cell line, normal kidney target cells tested simultaneously were not affected (Table 1, experiment 3). Thus the serum-induced lymphocyte cytotoxicity appeared to be antigen-specific (Table 2). Serum with LDA-like activity from patient T5 did not cause a significant reduction of either TCC-derived target cells or the normal kidney cells, in the absence of lymphocytes (Table 1, experiments 2 and 3).

While direct intrinsic lymphocyte mediated cytotoxicity against TCC target cells is also demonstrated [for example, lymphocytes from T1 versus lymphocytes from C2, in the presence of normal serum from C1, tested against TCC-bladder target cells (Table 1, experiment 3)], our method of analysis takes into account (by appropriate controls) the intrinsic cytotoxicity caused by the lymphocytes themselves; and thus the CI's (Table 1) are an estimation of the cytotoxicity induced by the presence of the LDA-like activity in serums from patients T5 and T6.

Studies to define target cell specificity were carried out with serum from patient T5. The results of 18 independent experiments demonstrate that serum from patient T5 (diluted 1 : 5) induced cytotoxicity in lymphocytes obtained from 87 percent of the TCC and control donors against the three TCC-derived target cell lines tested (Table 2). In addition, no cytotoxicity induction was detected against any of the normal target cells lines or against renal cell carcinoma tested simultaneously. Nine serum samples obtained at different dates from patients T5 and T6 also showed similar LDA-like activity in assays not presented here.

The cytotoxicity inducing activity of the serum factor (or factors) described above resembles the activity of lymphocyte dependent antibodies reported previously. Neither requires added complement, nor is either effective in the absence of effector (lymphoid) cells. Like previously described LDA (*3*), the serum factor demonstrated above appears to determine the specificity of the induced cytotoxicity. The LDA-like activity of serums from patients T5 and T6 appears to be directed against TCC (and not against HL-A antigens) since the cytotoxicity was induced against all the TCC cell lines tested but not against any of the seven non-TCC control cell lines (Table 2). While such target cell specificity suggests the participation of antibody, additional study will be required to determine whether this LDA-like factor is an IgG immunoglobulin.

It is not known whether thymus independent lymphocytes are the effector cells in these studies as they are in other LDA assays. This is significant because it has been reported recently that thymus independent lymphocytes are important in cell mediated cytotoxicity against human TCC-bladder cells (*12*). Thus, if the LDA-like activity of the TCC serums is similar to previously described LDA, it may potentiate the cytotoxicity of an effector cell type important in cell mediated immunity to human TCC.

The demonstration of the existence of serum dependent lymphocyte cytotoxicity against TCC's may provide an additional avenue for investigating the development and expression of immune responses against human solid tumors. If serum factors are capable of inducing in vivo lymphocyte mediated tumor destruction in man as they have been reported to do in animals (*13*), then perhaps the opportunity for immunotherapy in patients with transitional cell carcinomas may be enhanced.

THOMAS R. HAKALA
PAUL H. LANGE
Department of Surgery/Urology, Veterans Administration Hospital, Minneapolis, Minnesota 55417, and *Department of Urologic Surgery, University of Minnesota, Minneapolis 55455*

References and Notes

1. K. Hellström and I. Hellström, *Adv. Immunol.*, in press.
2. P. Perlmann, H. Perlmann, H. Wigzell, *Transplant. Rev.* **13**, 91 (1972).
3. I. MacLennan, *ibid.*, p. 67.
4. J. Bubenik, P. Perlmann, K. Helmstein, G. Moberger, *Int. J. Cancer* **5**, 310 (1970).
5. I. Hellström, K. Hellström, H. Sjögren, G. Warner, *ibid.* **7**, 1 (1971).
6. T. Hakala, A. Castro, A. Elliott, E. Fraley, *J. Urol.*, in press.
7. J. Bubenik *et al.*, *Int. J. Cancer*, in press.
8. A. Boyum, *Scand. J. Clin. Lab. Invest.* **21** (Suppl. 97), 77 (1968).
9. A. Coulson and D. Chalmers, *Lancet* **1964-I**, 468 (1964).
10. T. Greenwalt, M. Gajewski, J. McKenna, *Transfusion* **2**, 221 (1962).
11. W. Goyle, *ibid.* **6**, 761 (1968).
12. C. O'Toole, P. Perlmann, H. Wigzell, B. Unsgaard, C. Zetterlund, *Lancet* **1973-I**, 1085 (1973).
13. P. Hersey, *Nat. New Biol.* **244**, 22 (1973).
14. Supported in part by research funds of the Veterans Administration, PHS grant CA 13095-02, and PHS training grant in urology AM 05514-08. P.H.L. is a clinical fellow of American Cancer Society. We thank J. Johnson, E. Arfman, and N. Keldahl for technical assistance.

11 January 1974

Partition of Tissue Functions in Epithelia: Localization of Enzymes in "Mitochondria-Rich" Cells of Toad Urinary Bladder

Abstract. *The mucosal epithelium of the toad urinary bladder reabsorbs sodium, acidifies the urine, and is responsive to neurohypophyseal hormones. Mucosal epithelial cells, consisting of two major morphologic cell types, "mitochondria-rich" and "granular," were removed from the bladder and separated by density gradient centrifugation. The mitochondria-rich cells contained three times as much carbonic anhydrase activity as the granular cells. Oxytocin caused a 235 percent increase in the adenosine 3',5'-monophosphate content of mitochondria-rich cells but had no effect on the granular cells. The evidence indicates that the mitochondria-rich cell, which accounts for only 15 percent of the mucosal cells, plays a major role in the mediation of sodium ion and hydrogen ion transport in the toad bladder and is a specific site of action of neurohypophyseal hormones.*

Epithelial membranes often contain several types of cells, each of which may contribute to different aspects of the tissues' overall physiology. Oxytocin, vasopressin, and related neurohypophyseal hormones cause an increase in the hydroosmotic permeability (*1*) of the urinary bladder of the toad, *Bufo marinus*, and an increase in the rate at which the tissue transports sodium (*2*) from the luminal surface to the nutrient surface. These responses are evidently mediated by a hormone-sensitive adenylate cyclase (*3*, *4*).