IV.
Results

PART ONE: STUDIES OF PREPARED EXTRACTS

I: Extracts' Total Protein Content

The total protein content of prepared extracts, measured using the standard Folin method, is depicted in Table III.

II: Control Level of Leukocyte Adherence

Since serum (FC or human) has nonspecific depressing effect on cell adherence (Halliday, 1979 and Seddek, 1979), all experiments were done using serum-free RPMI-1640 medium. To estimate the basal level of % adherent cells in protein-free medium, using the haemocytometer LAI version, two groups of PBL were tested (Table IV). Group A of 12 control subjects, gave % adherent cells ranging from 70.0 to 90.0 with a mean of 80.0 ± 2.2 No difference was recorded between response of control patients with benign bilharzial granuloma (BGr, last 2 samples) and the other healthy controls.

Group B, of 6 patients with stage II or III urinary bladder squamous cell carcinoma (SqCC/UB) showed % adherent cells ranging between 66.6 and 87.0 with a mean of 75.0 ± 3.8.

The two means showed no difference and these adherence levels were considered as the basis for evaluating the effect of added extracts.

TABLE III

TOTAL PROTEIN CONTENT OF PREPARED EXTRACTS

Type of Extract*	No.**	Total Protein (ug/ml)
SqCC/UB	1/80	3500
SqCC/UB	1/82	9200
SqCC/UB	2/82	7500
SqCC/UB	3/82	5400
TrCC/UB	1/80	6750
AdnC/UB	1/80	1350
NorE/UB	1/82	850
SqCC/CX	1/82	6700
Shaem/SEA	1/83	3650

* Type of extract: referes to type of tissue and site of origen e.g. SqCC/UB is a squamous cell carcinoma of the urinary bladder.

** No.: indicates the serial number of the extract within the same type of extracts and the year of preparation.

TABLE IV

LEUKOCYTE ADHERENCE FOLLOWING INCUBATION IN PROTEIN-FREE MEDIUM

Exp. Gp.	Leukocytes	Adherent cells (% ± SE)	Mean of adherent cells (% ± SE)
A	Normal	88.7 ± 0.8	80.0 ± 2.2
		72.7 ± 2.3	
		77.3 ± 0.6	
		86.8 ± 0.6	
		85.6 ± 1.7	
		71.7 ± 2.7	
		74.3 ± 1.2	
		75.2 ± 1.4	
		78.3 ± 1.3	
		90.0 ± 2.8	
	BGr/UB	70.0 ± 2.5	
		89.2 ± 1.0	
B	SqCC/UB	75.7 ± 0.6	75.0 ± 3.8
		68.4 ± 1.1	(P > 0.05)
		87.0 ± 0.8	
		80.9 ± 1.0	
		77.6 ± 1.1	
		60.6 ± 2.2	

III: Extracts' Toxicity in LAI Test

The nonspecific or toxic effect of tumor and control extracts was examined in the haemocytometer version of the LAI test. Three different concentrations of extracts' preparations (250, 500 and 100 ug/ml) were used in this study except for the Shaem/SEA1/83 extract which was used at 25,50,100 ug/ml concentrations. All prepared extracts were exposed to PBL collected from blood bank donors and/or from healthy laboratory personnel (Table V a,b). The experiments depend upon comparing results obtained at each concentration of any of the extracts to those observed when the control PBL were tested in absence of any antigenic material.

Using protein-free medium (zero), PBL from the control subjects expressed % adherence in the range of 90.6 to 73.4 (Table Va) with a mean value of 83.4 ± 0.14 (not in tables). This 83.4 ± 0.14 mean value was considered as a blank value for comparing all other results. Regardless of the extract types used, a decrease in percentage of adherent cells was observed with increase of extracts' concentration (Table Va). This observations appears clear on examining the mean values plotted in Table Vb.

When mean of % adherent cells following testing each of the nine extracts, with control PBL, were compared to the blank value (83.4 ± 0.14) it appeared that 250 ug/ml is the suitable nontoxic concentration for all tested tissue extracts. On the other hand, 25 ug/ml was the suitable concentration for Shaem/SEA1/83 extract.

TABLE V

RELATIONSHIP BETWEEN EXTRACTS CONCENTRATION AND ADHERENCE OF CONTROL LEUKOCYTES

(a)

Extract	Adherent cells (%±SE) with different extracts concentrations ug/ml			
	zero	250	500	1000
SqCC/UB1/80	85.5 ± 1.3	78.4 ± 1.1	39.0 ± 1.0	-----
	84.5 ± 1.0	73.2 ± 1.8	62.5 ± 0.9	-----
	77.2 ± 1.8	78.0 ± 2.3	-----	-----
	90.6 ± 0.5	70.9 ± 1.6	40.8 ± 1.3	-----
	81.0 ± 1.2	74.6 ± 1.0	49.1 ± 2.6	-----
SqCC/UB1/82	82.6 ± 1.1	77.2 ± 1.8	40.3 ± 1.4	22.4 ± 1.7
	85.7 ± 1.3	71.9 ± 1.4	51.8 ± 1.8	39.0 ± 1.0
	81.8 ± 1.4	66.4 ± 2.3	62.3 ± 1.4	-----
	87.6 ± 1.0	-----	57.5 ± 2.0	35.2 ± 0.8
SqCC/UB2/82	88.8 ± 0.9	71.5 ± 1.8	65.1 ± 1.5	40.8 ± 1.3
	73.4 ± 0.7	79.7 ± 2.1	68.9 ± 1.3	22.4 ± 1.7
	80.7 ± 1.3	77.2 ± 2.0	56.0 ± 1.2	49.1 ± 2.6
	89.2 ± 0.9	89.2 ± 0.9	68.7 ± 2.2	48.8 ± 1.7
SqCC/UB3/82	86.2 ± 0.8	76.7 ± 2.1	46.7 ± 2.2	31.0 ± 0.6
	82.6 ± 1.0	78.0 ± 1.4	59.9 ± 2.9	44.7 ± 0.9
	77.2 ± 1.9	80.7 ± 1.3	55.8 ± 1.5	-----
TrCC/UB1/80	85.5 ± 1.3	79.6 ± 1.6	68.0 ± 1.8	-----
	85.7 ± 0.9	74.0 ± 1.8	65.7 ± 2.5	59.9 ± 2.9
	84.5 ± 1.0	81.9 ± 1.3	46.7 ± 3.8	32.4 ± 1.6
	90.6 ± 0.5	88.8 ± 0.9	51.1 ± 2.9	40.1 ± 1.3
AdnC/UB1/80	85.5 ± 1.3	78.4 ± 1.1	57.5 ± 2.0	22.4 ± 1.7
	84.5 ± 1.0	70.9 ± 1.6	51.8 ± 1.8	16.0 ± 0.4
	77.2 ± 1.8	66.5 ± 1.4	39.0 ± 1.0	18.0 ± 0.7
NorE/UB1/82	88.8 ± 0.9	86.8 ± 1.3	79.8 ± 1.2	-----
	73.4 ± 0.7	89.5 ± 2.1	91.3 ± 1.6	-----
	80.7 ± 1.3	70.9 ± 1.6	88.7 ± 0.9	-----
	89.2 ± 0.9	70.1 ± 0.9	83.1 ± 1.4	-----
SqCC/CX1/82	86.2 ± 0.8	79.7 ± 2.0	68.8 ± 1.0	46.4 ± 1.7
	82.6 ± 1.0	70.4 ± 1.2	73.0 ± 2.1	34.0 ± 0.8
	77.2 ± 1.9	70.9 ± 1.0	69.3 ± 1.5	-----

Extract	Adherent cells (%+SE) with different extracts concentrations ug/ml			
	zero	25	50	100
Shaem/SEA1/83	78.4 ± 1.1	71.0 ± 0.7	76.1 ± 2.5	67.5 ± 2.1
	87.0 ± 0.6	80.7 ± 1.3	81.5 ± 4.6	63.4 ± 1.8
	79.9 ± 1.3	90.2 ± 1.7	75.5 ± 4.9	60.9 ± 1.0
	83.0 ± 0.8	75.6 ± 1.7	82.0 ± 0.8	70.3 ± 1.7

TABLE V

(b)

Extract	Adherent cells (%±SE)* with different extracts concentrations ug/ml			
	zero	250	500	1000
SqCC/UB1/80	83.8 ± 2.2	75.0 ± 1.5 (P >0.05)	47.9 ± 4.5 (P <0.01)	-----
SqCC/UB1/82	84.4 ± 1.4	71.8 ± 3.1 (P >0.05)	53.0 ± 2.4 (P <0.01)	32.2 ± 5.0 (P <0.01)
SqCC/UB2/82	83.0 ± 1.9	79.4 ± 1.8 (P >0.05)	64.7 ± 4.7 (P <0.05)	40.3 ± 3.1 (P <0.01)
SqCC/UB3/82	82.0 ± 3.8	78.5 ± 3.7 (P >0.05)	54.1 ± 3.0 (P <0.01)	37.9 ± 6.3 (P <0.01)
TrCC/UB1/80	86.6 ± 1.4	81.1 ± 3.1 (P >0.05)	57.9 ± 5.3 (P <0.05)	44.1 ± 8.2 (P <0.01)
AdnC/UB1/80	82.4 ± 2.6	71.9 ± 3.5 (P >0.05)	49.4 ± 5.5 (P <0.01)	18.8 ± 1.9 (P <0.01)
NorE/UB1/82	83.0 ± 3.8	79.3 ± 5.1 (P >0.05)	85.7 ± 2.6 (P >0.01)	-----
SqCC/CX1/82	82.0 ± 2.6	73.7 ± 3.0 (P >0.05)	70.4 ± 1.3 (P <0.05)	40.2 ± 6.2 (P <0.01)
Extract	Adherent cells (%+SE)* with different extracts concentrations ug/ml			
	zero	25	50	100
Shaem/SEA1/83	82.1 ± 1.9	79.4 ± 4.1 ((P >0.05)	78.8 ± 1.7 (P >0.05)	65.5 ± 2.1 (P <0.05)

* Mean values of data in Table Va

IV: Extracts Specific Reactivity

After determining the nontoxic concentration of the prepared extracts and for evaluation of specificity, and extent of the LAI response, testing was expanded to study response of leukocyte preparations of patients with different types of urinary bladder carcinomas (namely squamous cell-, transitional cell- and adenocarcinomas) and control donors (including cervical squamous cell carcinoma and bilharzial patients). This investigation included three separate sets of experiments using the haemocytometer version of the LAI test.

In the first series, the 4 urinary bladder squamous cell carcinoma extracts (SqCC/UB1/80, SqCC/UB1/82, SqCC/UB2/82, SqCC/UB3/82) were tested against PBL samples of control donors or patients with squamous cell carcinoma of urinary bladder (Table VI). Actually this series has three different control parameters. First, determination of the level of adherence of control PBL in absence of any protein material. Second, estimation of the effect of SqCC/UB extracts on adherence of control PBL (using the nontoxic 250 ug/ml concentration). The third control parameter included the level of adherence of SqCC/UB PBL in absence of SqCC/UB extracts. These distinict control conditions, showed similar levels of adherence (invidual data and mean values of adherence expelled in table VI).

Four test groups are the groups in which PBL of SqCC/UB patients were exposed to LAI testing in presence of 250 ug/ml of each

TABLE VI

SPECIFIC REACTIVITY OF SqCC/UB EXTRACTS

Extracts	Leukocytes	Adherent cells (% ± SE)		Mean adherent cells (% ± SE)	
		zero	250*	zero	250*
SqCC/UB1/80	Con	78.2 ± 2.7 87.6 ± 1.8 81.1 ± 2.5	67.6 ± 3.6 85.7 ± 2.1 77.2 ± 3.9	82.3 ± 2.8	76.8 ± 5.2
	SqCC/UB	89.3 ± 1.5 77.9 ± 2.1 80.3 ± 2.7	54.8 ± 1.7 35.9 ± 2.3 51.6 ± 1.7	82.5 ± 3.5	47.4 ± 5.8 (P <0.01)
SqCC/UB1/82	Con	87.6 ± 2.0 84.6 ± 2.2 72.6 ± 3.0	78.1 ± 2.7 71.8 ± 1.9 67.6 ± 3.6	81.6 ± 4.6	72.8 ± 2.8
	SqCC/UB	89.3 ± 1.5 87.6 ± 2.0 86.6 ± 1.8 78.2 ± 2.1	50.2 ± 1.9 26.1 ± 1.5 46.0 ± 1.7 30.4 ± 2.0	85.4 ± 2.5	38.2 ± 5.9 (P <0.01)
SqCC/UB2/82	Con	87.6 ± 2.0 84.6 ± 2.2 72.6 ± 3.0	80.3 ± 2.4 78.4 ± 2.1 66.5 ± 2.9	81.6 ± 4.6	75.1 ± 4.3
	SqCC/UB	89.3 ± 1.5 77.9 ± 2.1 80.3 ± 2.7	79.4 ± 2.4 73.6 ± 2.4 79.5 ± 0.6	82.5 ± 3.5	77.5 ± 2.0 (P >0.05)
SqCC/UB3/82	Con	93.6 ± 1.2 90.4 ± 1.8 92.2 ± 1.4	88.2 ± 1.3 85.9 ± 1.7 83.5 ± 1.8	92.1 ± 0.9	85.9 ± 1.4
	SqCC/UB	85.3 ± 1.9 88.8 ± 2.0 80.6 ± 2.0 84.9 ± 2.1	58.2 ± 2.4 64.2 ± 2.3 55.0 ± 1.8 55.3 ± 2.0	84.9 ± 1.7	58.2 ± 2.1 (P <0.01)

* The nontoxic concentration of extracts in ug/ml as previously determined

of the 4 above mentioned SqCC/UB extracts each separately. Specific adherence inhibition was observed in presence of SqCC/UB1/80, SqCC/UB1/82 and SqCC/UB3/82. However, the SqCC/UB2/82 was found to be incapable of elisiting reduction in adherence of sensitized leukocytes.

The data of the second set of experiments, illustrated in Table VII, show the level of specificity of LAI using 4 control extracts. PBL obtained from control donors showed % adherence in the range of 93.6 to 75.3 in absence of any extract. The mean percentage of adherent cells when PBL of normal donors or of SqCC/UB patients, were exposed to either zero or 250 ug of extract of transitional cell carcinoma of the urinary bladder (TrCC/UB1/80), adenocarcinoma of the urinary bladder (AdnC/UB1/80), normal urinary bladder tissue (NorE/UB1/82) or squamous cell carcinoma of the cervix (SqCC/CX1/82). On the other hand, the TrCC/UB1/80, AdnC/UB1/80 and SqCC/CX1/82 extracts induced strong, LAI specifically when reacted with PBL of patients sensitized to their tumor type (Table VII).

The Shaem/SEA1/83 extract was tested in the third set of experiments (Table VIII) against 3 groups of PBL. First group comprised 5 PBL samples obtained from normal control donors. The second included 5 PBL samples of Shaem patients. Three PBL samples delivered from SqCC/UB patients consisted the third experimental group.

Regardless of type of leukocyte donors, no adherence inhibition was observed in absence of the Shaem extract. The Shaem

TABLE VII

SPECIFIC REACTIVITY OF DIFFERENT CONTROL EXTRACTS

Extract	Leukocytes	Adherent cells (% ± SE)		Mean adherent cells (% ± SE)	
		zero	250	zero	250
TrCC/UB1/80	Con	78.1 ± 2.2 75.3 ± 3.6 79.0 ± 1.8	67.7 ± 2.3 69.8 ± 3.9 71.0 ± 2.4	77.5 ± 1.1	72.5 ± 2.1
	TrCC/UB	73.4 ± 2.2 82.0 ± 2.6 80.7 ± 2.4	36.4 ± 2.3 57.3 ± 3.1 45.7 ± 3.6	78.7 ± 2.7	46.5 ± 6.0 (P <0.01)
	SqCC/UB	77.0 ± 2.1 81.1 ± 1.3 88.6 ± 1.7 90.4 ± 1.8 87.6 ± 2.0	71.4 ± 4.1 72.6 ± 3.0 78.2 ± 2.7 77.2 ± 3.5 78.1 ± 2.7	84.9 ± 2.5	75.5 ± 1.5
AdnC/UB1/80	Con	78.1 ± 2.2 75.3 ± 3.6 79.0 ± 1.8	68.7 ± 3.2 68.2 ± 3.5 66.5 ± 2.9	77.5 ± 1.1	67.8 ± 0.7
	AdnC/UB	76.7 ± 2.3 77.9 ± 2.1 90.7 ± 1.4	37.8 ± 2.6 53.5 ± 3.2 39.1 ± 3.6	81.8 ± 4.5	43.5 ± 5.0 (P <0.01)
	SqCC/UB	73.4 ± 2.2 79.4 ± 2.1 73.9 ± 1.9	56.1 ± 2.6 78.0 ± 2.0 67.6 ± 3.1	75.6 ± 1.9	70.2 ± 3.9
NorE/UB1/82	Con	89.3 ± 1.5 90.4 ± 1.8 93.6 ± 1.2	88.1 ± 1.4 81.2 ± 1.7 89.3 ± 1.5	91.1 ± 1.3	86.2 ± 2.5
	SqCC/UB	73.4 ± 2.2 79.4 ± 2.1 73.9 ± 1.9	83.7 ± 1.8 77.0 ± 2.0 71.0 ± 1.8	75.6 ± 1.9	77.2 ± 3.7
SqCC/CX1/82	Con	89.3 ± 1.5 90.4 ± 1.8 93.6 ± 1.2	79.5 ± 3.0 78.0 ± 2.0 80.6 ± 2.0	91.1 ± 1.3	79.4 ± 0.8
	SqCC/CX	75.8 ± 2.5 93.6 ± 1.2 89.2 ± 1.9	67.2 ± 5.1 73.2 ± 2.9 74.4 ± 3.4	86.2 ± 5.4	71.6 ± 1.9 (P <0.05)
	SqCC/UB	73.4 ± 2.2 79.4 ± 2.1 73.9 ± 1.9	66.7 ± 2.8 68.4 ± 2.6 69.7 ± 4.1	75.6 ± 1.9	68.3 ± 0.9

TABLE VIII

SPECIFIC REACTIVITY OF Shaem/SEA

Extract	Leukocytes	Adherent cells (% ± SE)		Mean adherent cells (% ± SE)	
		zero	25*	zero	25*
Shaem/SEA/83	Con	77.6 ± 3.0	75.3 ± 1.9	82.8 ± 2.6	74.2 ± 2.4
		80.3 ± 2.7	78.7 ± 1.4		
		78.2 ± 2.7	64.9 ± 2.9		
		87.6 ± 1.0	76.1 ± 4.1		
		90.4 ± 1.8	75.8 ± 2.5		
	Shaem	85.7 ± 2.1	68.4 ± 4.5	85.3 ± 1.6	50.6 ± 5.9
		89.3 ± 1.5	57.3 ± 3.3		(P <0.01)
		80.3 ± 2.7	59.3 ± 2.5		
		87.6 ± 2.0	39.2 ± 3.7		
		83.8 ± 2.2	48.6 ± 3.5		
	SqCC/UB	95.3 ± 1.1	83.7 ± 1.8	89.1 ± 4.9	79.9 ± 6.9
		92.6 ± 1.6	89.4 ± 1.6		
		79.4 ± 2.4	66.5 ± 2.9		

* The nontoxic concentration of extract in ug/ml as previously determined

extract, did not affect also the adherence of leukocytes of normals or SqCC/UB patients. On the other hand, the Shaem extract induced a great lowering of adherence of Shaem sensitized leukocytes (50.6 + 5.9% mean adherence). These observations point to the absence of cross-creativity between SqCC/UB and Shaem and emphasise on the high specificity of Shaem/SEA1/83 extract in the LAI test.

V. Purification of Tumor Extracts

Gel filtration chnomatography was used to fractionate the reactive SqCC/UB1/82 extract for isolation of the reactive fraction and to determine its molecular weight.

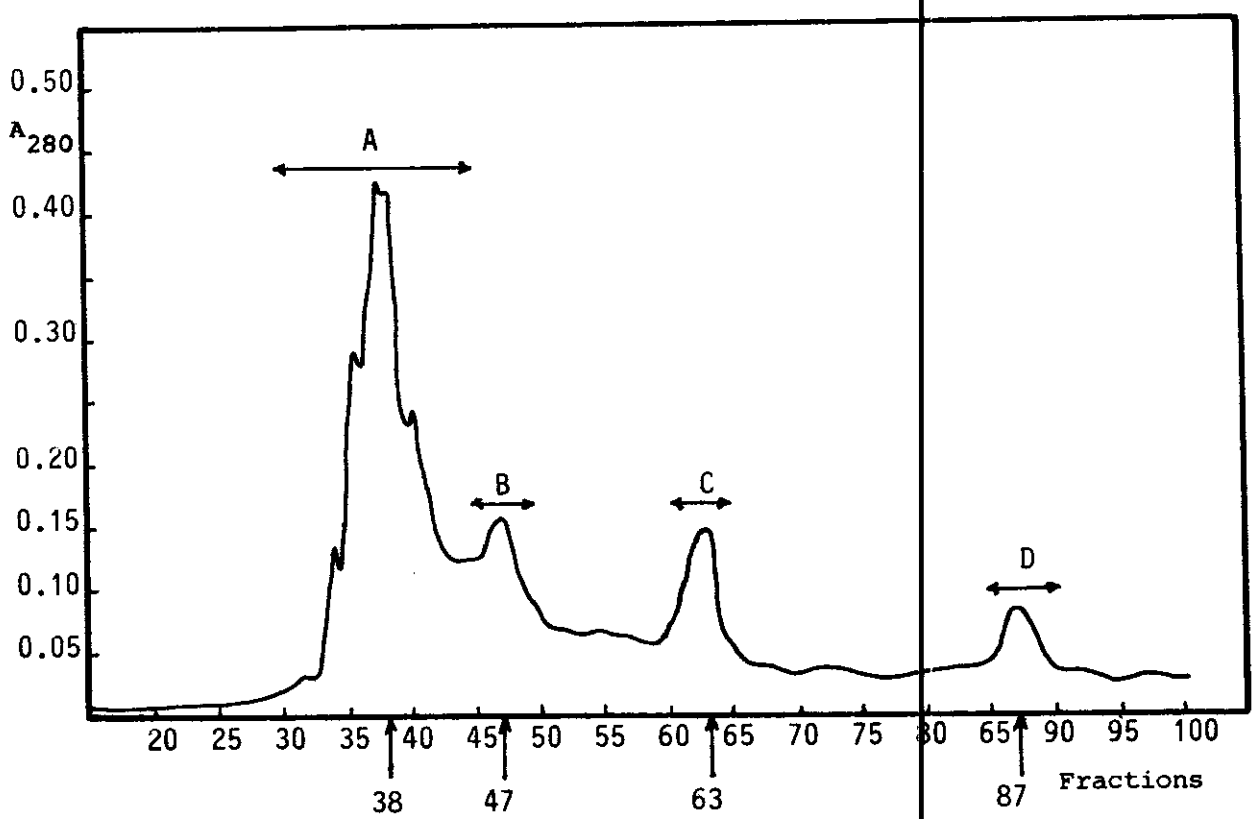
A. Isolation of the reactive fraction

Figure I showes a typical elution profile of the reactive SqCC/UB1/82 antigenic extract from 1.6x80 cm column of Sephacryle S-200. The elution profile shows 4 major peaks; A, B, C and D. Fractions under each of the 4 mentioned peaks were collected, pooled and tested for specific reactivity, as well as the crude unfractionated , extract, using the haemocytometer LAI against 3 PBL samples obtained from 3 early stage SqCC/UB patients (Table IX).

Values of % adherent cells in presence of the crude SqCC/UB1/82 extract were 38.0, 42.0 and 30.0 with a mean of 37.0 ± 1.3%. PBL samples when tested in presence of peacks A,B or D materials showed a mean adherence of 81.2 ± 1.8%, 76.7 ± 2.1 % and 83.3 ± 1.9%. By testing the same PBL samples with the peack C material of the

FIGURE I

ELUTION PROFILE OF SqCC/UB1/82 ANTIGENIC EXTRACT



Fractionation of the SqCC/UB1/82 reactive antigenic extract was performed on Sephacryl S-200 column (1.6 x 85 cm). Elution was done using PBS, pH 7.2, monitored at 280 nm (0.5 range) and collected in 2 ml fractions.

TABLE IX

EFFECT OF ISOLATED FRACTION OF EXTRACT ON ADHERENCE OF LEUKOCYTES

Extract*	Adherent cells (% ± SE)	Mean adherent cells (% ± SE)
SqCC/UB1/82	38.0 ± 1.2 42.8 ± 1.3 30.0 ± 1.3	37.0 ± 1.3
SqCC/UB1/82 (peack A)	80.1 ± 1.7 74.6 ± 1.4 89.0 ± 2.0	81.2 ± 1.8 (P >0.01)
SqCC/UB1/82 (peack B)	74.9 ± 2.3 75.0 ± 1.4 80.3 ± 2.7	76.7 ± 2.1 (P >0.01)
SqCC/UB1/82 (peack C)	21.0 ± 1.1 30.0 ± 1.1 13.0 ± 1.3	21.3 ± 1.2 (P <0.05)
SqCC/UB1/82 (peack D)	90.1 ± 1.2 81.0 ± 2.3 78.9 ± 1.7	83.3 ± 1.9 (P >0.01)

* SqCC/UB1/82 extract as well as SqCC/UB1/82 isolated peaks were all tested at the same concentration of 250 ug/ml

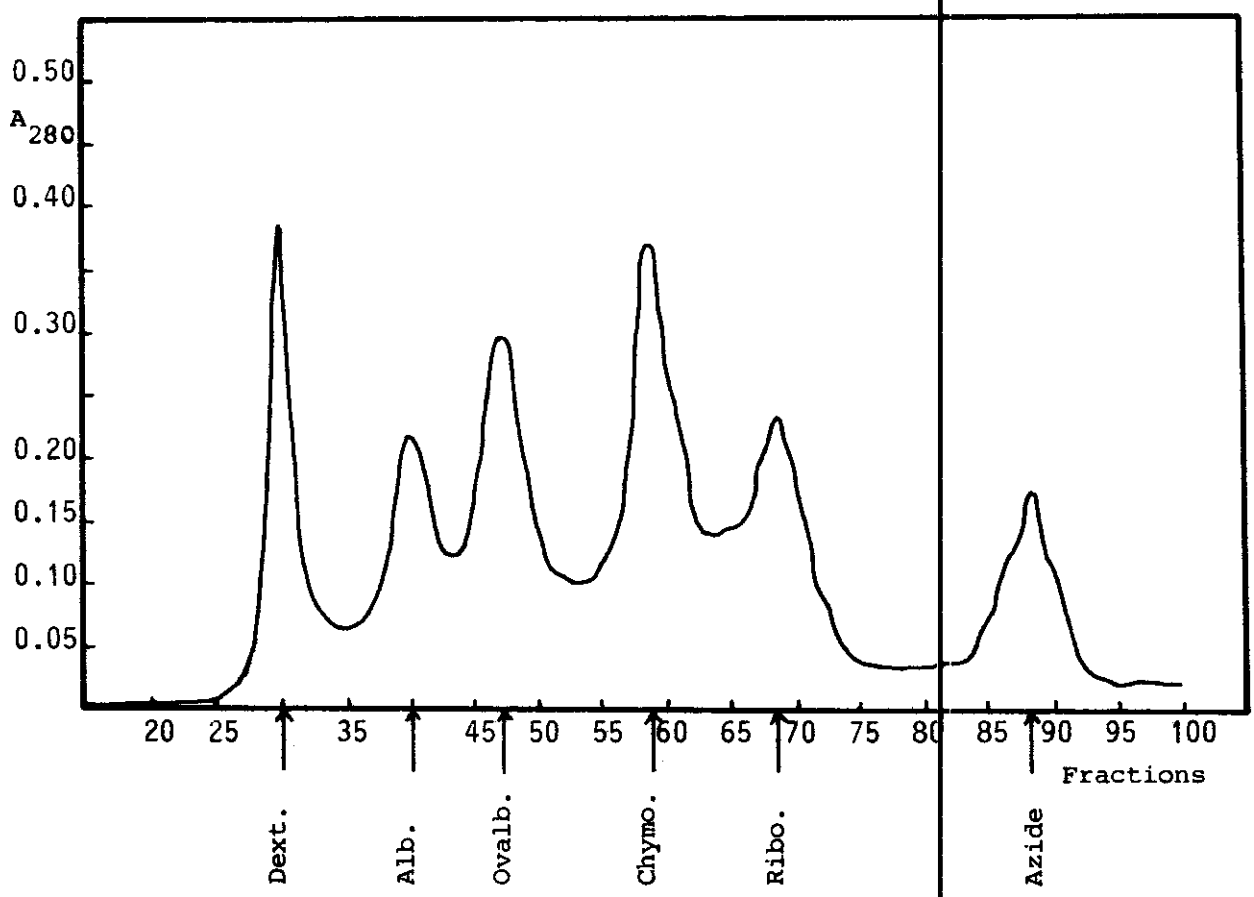
SqCC/UB1/82 antigenic extract, percentage adherence were 21.0, 30.0 and 13.0. The mean adherence was 21.3 ± 1.2 %.

B: Molecular weight determination

A mixture of standard proteins with known molecular weight were fractionated on Sephacryl S-200 standard column and their elution volumes V_e were determined from their elution profile Figure II. The void volume V_o was determined using blue Dextran 2000 and the total bed volume V_t was estimated using sodium azid solution. Values of K_{av} [$K_{av} = (V_e-V_o)/(V_t-V_o)$] for each of the standard proteins were calculated. A standard curve between Kav values and log molecular weight was plotted in Figure III. From this standard curve and according to the calculated K_{av} for the reactive fraction (peack C) of the SqCC/UB1/82 extract, the corresponding log molecular weight value was determined (4.3). Accordingly the molecular weight of this active fraction was predicted and found to be 16,227 daltons.

FIGURE II

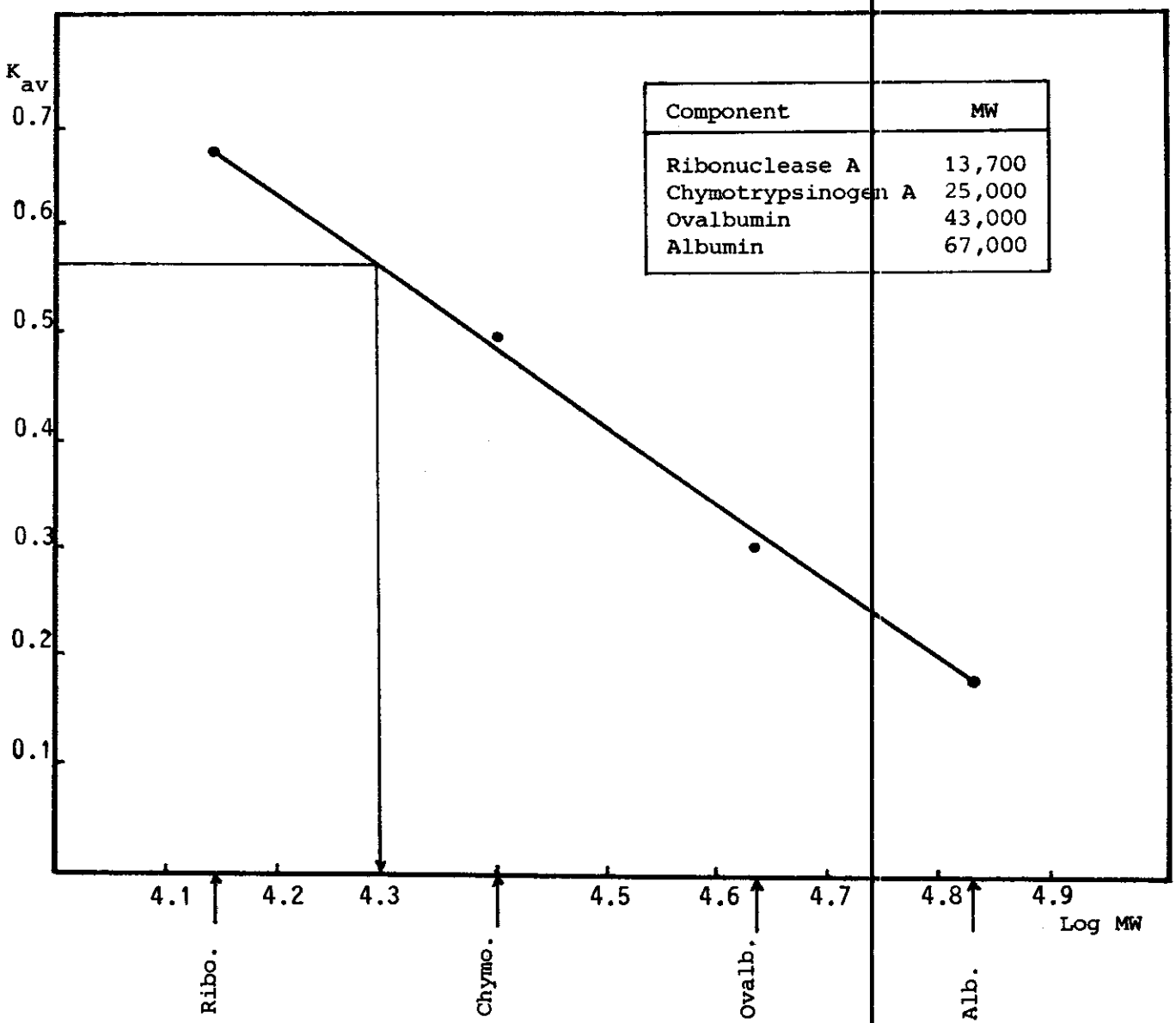
ELUTION PROFILE OF STANDARD PROTEINS



Standard proteins of known molecular weights were chromatographed on the same Sephacryl S-200 column (standard column). Elution and monitoring was performed typically as done with the SqCC/UB1/82 antigenic extract.

FIGURE III

MOLECULAR WEIGHT STANDARD CURVE



This standard curve is presented on a semilogarithmic paper (MW scale). A positive correlation coofficient [$r=(n\Sigma xy-\Sigma x\Sigma y)/n^2S'S''$] of 0.98 (98% positive correlation) is expressed by the data plotted in the curve.

PART TWO: LAI ASSAY VERSIONS

I. Reproducibility and Reliability of LAI Assay Versions

Because of the availability of using different LAI assay verions in this study, it was a must to compare between these versions. Four different extracts were chosen; NorE/UB1/82, Shaem/SEA1/83, SqCC/CX1/82 and SqCC/UB1/82 (Table X a,b).

Basically the level of adherence of PBMC, obtained from 3 control donors, 5 Shaem patients, 3 SqCC/CX patients and 3 patients with SqCC/UB, was determined in protein-free medium (none) using the haem-LAI, tube-LAI and micro-LAI versions. Individual values of % adherence, as shown in Table Xa, indicate no difference regardless the type of assay version. Mean values plotted in Table Xb make this observation easy to notic.

From the viewpoint of covering each piece of experiment with proper control conditions, PBMC from control donors were exposed to testing in presence of each of the above mentioned extracts. Beside the absence of any adherence inhibition effect of these extracts, the three versions showed reproducible and reliable results (Table Xb). The second control aproach is that of measuring the effect of each of NorE/UB, Shaem/SEA and SqCC/CX extracts on the adherence on PBMC of the patients of great interest in this work, namely the SqCC/UB patients. None of the extracts affected the adherence of these cells, in the haem-, tube- nor micro- LAI (Table Xb). In contrast, the

TABLE X

EVALUATION OF DIFFERENT LAI ASSAY VERSIONS

(a)

Extracts	Leukocytes	Haem-LAI Adherent cells (% ± SE)	Tube-LAI Adherent cells (% ± SE)	Micro-LAI Adherent cell/well (count ± SE)
None	Con	74.9 ± 3.5	86.7 ± 3.5	72.3 ± 3.2
		70.6 ± 2.3	81.8 ± 4.4	61.8 ± 2.3
		89.5 ± 2.2	92.2 ± 2.1	80.8 ± 5.7
	Shaem	73.2 ± 4.0	83.2 ± 3.1	67.3 ± 1.4
		80.1 ± 2.8	84.4 ± 3.6	73.0 ± 2.1
		69.8 ± 4.9	61.7 ± 5.8	75.7 ± 0.5
		90.2 ± 2.2	-----	99.3 ± 8.0
		83.5 ± 3.4	-----	72.3 ± 1.7
	SqCC/CX	78.4 ± 3.5	79.8 ± 1.7	76.5 ± 0.3
		79.1 ± 3.6	78.7 ± 3.0	88.3 ± 1.1
		84.1 ± 3.2	92.1 ± 1.8	68.0 ± 1.3
	SqCC/UB	88.9 ± 2.4	77.0 ± 2.0	76.0 ± 7.1
		75.8 ± 3.9	81.3 ± 3.1	86.5 ± 4.2
		79.4 ± 3.1	83.6 ± 3.8	93.3 ± 3.9
NorE/UB1/82	Con	82.0 ± 3.5	90.3 ± 1.2	77.0 ± 2.9
		83.8 ± 3.8	94.4 ± 1.6	70.0 ± 5.3
		71.7 ± 3.6	83.4 ± 1.1	87.0 ± 2.1
		87.9 ± 2.5	85.7 ± 2.1	80.0 ± 0.7
		89.6 ± 2.7	-----	77.5 ± 2.5
	SqCC/UB	74.4 ± 3.8	91.2 ± 1.9	76.0 ± 3.5
		80.9 ± 2.4	93.1 ± 0.8	68.5 ± 3.9
		82.0 ± 3.5	84.2 ± 4.0	85.0 ± 2.9
Shaem/SEA1/83	Con	76.5 ± 2.7	88.5 ± 0.4	78.0 ± 4.2
		82.0 ± 3.5	91.0 ± 1.5	84.5 ± 3.9
		69.4 ± 3.3	81.3 ± 3.1	75.3 ± 1.5
	SqCC/UB	73.2 ± 3.6	94.4 ± 1.6	74.0 ± 2.9
		72.6 ± 4.6	91.2 ± 1.9	85.0 ± 2.5
		83.8 ± 3.8	95.6 ± 0.9	76.0 ± 0.9
		75.1 ± 7.3	-----	-----
		68.6 ± 3.4	-----	-----
	Shaem	74.8 ± 3.6	93.7 ± 1.2	47.3 ± 3.1
		59.0 ± 4.9	79.8 ± 1.8	42.0 ± 2.2
		53.1 ± 3.7	77.0 ± 2.0	34.0 ± 2.4
		52.1 ± 4.4	61.7 ± 5.8	49.0 ± 7.2
		37.7 ± 2.6	82.4 ± 5.2	49.7 ± 2.8
		41.6 ± 5.0	-----	36.7 ± 1.4
		31.0 ± 2.6	-----	33.3 ± 2.5
		22.4 ± 3.6	-----	16.7 ± 1.4
		58.6 ± 3.8	-----	37.5 ± 1.1
SqCC/CX1/82	Con	86.7 ± 2.2	85.7 ± 3.3	74.0 ± 2.8
		80.8 ± 1.5	93.1 ± 0.8	75.0 ± 4.2
		79.4 ± 1.2	88.5 ± 1.4	-----
	SqCC/UB	76.7 ± 0.8	81.3 ± 3.1	80.5 ± 1.1
		78.2 ± 1.7	83.2 ± 3.1	-----
		75.2 ± 3.9	78.7 ± 3.0	76.0 ± 5.0
	SqCC/CX	48.8 ± 2.4	86.7 ± 3.5	25.5 ± 3.2
		42.8 ± 1.4	81.8 ± 4.4	17.0 ± 2.8
		55.7 ± 3.2	92.2 ± 2.1	21.5 ± 6.0
SqCC/UB1/82	Con	89.4 ± 2.6	84.4 ± 1.2	91.5 ± 6.7
		93.9 ± 2.8	95.5 ± 3.2	96.0 ± 2.0
		79.5 ± 1.7	90.3 ± 1.4	76.5 ± 1.9
	SqCC/UB	57.9 ± 6.0	77.0 ± 2.0	21.0 ± 5.7
		50.1 ± 2.9	-----	14.0 ± 1.4
		32.6 ± 4.6	61.7 ± 5.8	33.5 ± 1.1
		42.8 ± 1.4	80.4 ± 2.0	26.5 ± 3.9
		57.0 ± 5.9	25.9 ± 6.2	31.7 ± 2.8
		40.0 ± 6.1	81.8 ± 4.1	37.0 ± 1.5
		30.5 ± 5.5	17.6 ± 5.3	10.0 ± 2.1
		49.4 ± 3.3	81.8 ± 4.4	50.7 ± 3.2
		35.6 ± 5.5	25.9 ± 6.2	27.7 ± 0.8
		34.4 ± 5.5	79.8 ± 1.7	11.0 ± 0.7
		47.6 ± 4.3	93.9 ± 2.8	-----

TABLE X

(b)

Extract	Leukocytes	Haem-LAI Adherent cells (% ± SE)	Tube-LAI Adherent cells (% ± SE)	Micro-LAI Adherent cell/well (count ± SE)
None	Con	84.0 ± 4.6	86.9 ± 3.0	78.3 ± 3.0
	Shaem	79.4 ± 3.6	76.4 ± 7.4	77.5 ± 5.6
	SqCC/CX	80.5 ± 1.8	83.5 ± 4.3	75.9 ± 4.4
	SqCC/UB	81.4 ± 3.9	80.6 ± 1.9	85.3 ± 5.0
NorE/UB1/82	Con	83.0 ± 3.1	88.5 ± 2.5	78.3 ± 2.7
	SqCC/UB	79.1 ± 2.4	89.5 ± 2.7	76.5 ± 4.8
Shaem/SEA1/83	Con	76.0 ± 3.7	86.9 ± 2.9	79.3 ± 2.7
	SqCC/UB	74.7 ± 2.5	93.7 ± 1.3	78.3 ± 2.0
	Shaem	44.8 ± 4.2 ($P < 0.01$)	78.9 ± 5.2 ($P > 0.05$)	38.5 ± 3.4 ($P < 0.01$)
SqCC/CX1/82	Con	82.3 ± 2.2	89.1 ± 2.2	74.5 ± 0.5
	SqCC/UB	76.7 ± 0.9	81.1 ± 1.3	78.3 ± 2.3
	SqCC/CX	49.1 ± 3.7 ($P < 0.05$)	86.9 ± 3.0 ($P > 0.05$)	21.3 ± 2.5 ($P < 0.01$)
SqCC/UB1/82	Con	87.6 ± 4.3	90.1 ± 3.2	88.0 ± 5.9
	SqCC/UB	43.5 ± 2.9 ($P > 0.05$)	62.6 ± 9.0 ($P < 0.01$)	26.2 ± 4.0 ($P > 0.01$)

SqCC/UB1/82 extract elicited a remarkable adherence inhibition on PBMC from SqCC/UB patients while using the three LAI-assay versions (Table X a,b), thus pointing to equal reliability and sensitivity of the three LAI versions.

A striking strange observation was, however, observed in cases of specific reactivity between the Shaem and SqCC/CX PBMC and their sensitizing antigenic extracts. There was detectable adherence inhibition in these cases while using the haem- and micro-LAI but not with the tube-LAI version. The tube method seemed to us nonreproducible and unreliable (Table Xb).

II. Colorimetric Modification of LAI-Microassay

A. MTT/Microcolorimetric-LAI test

As MTT is used in measuring the viability of cells in colorimetric microcytotoxicity assay, the technique was adapted to measure the adherence of cells in a micro-LAI assay. The standard curve between cell number/well and absorbance at 540 nm was estimated using a double fold dilution of PBMC of control donor, starting from a concentration of $100x10^3$ cells/well. A positive correlation coofficient of 0.94 (94% positive correlation) was expressed by the data plotted in the curve (Figure IV).

This modified LAI-assay was used to demonstrate LAI of 2 groups of PBMC (Table XI). Three PBMC samples from control donors showed absorbance values of 0.74, 0.80 and 0.76 in absence of any

FIGURE IV

CORRELATION BETWEEN PBMC COUNT AND MEASURED ABSORBANCE

USING MTT STAIN

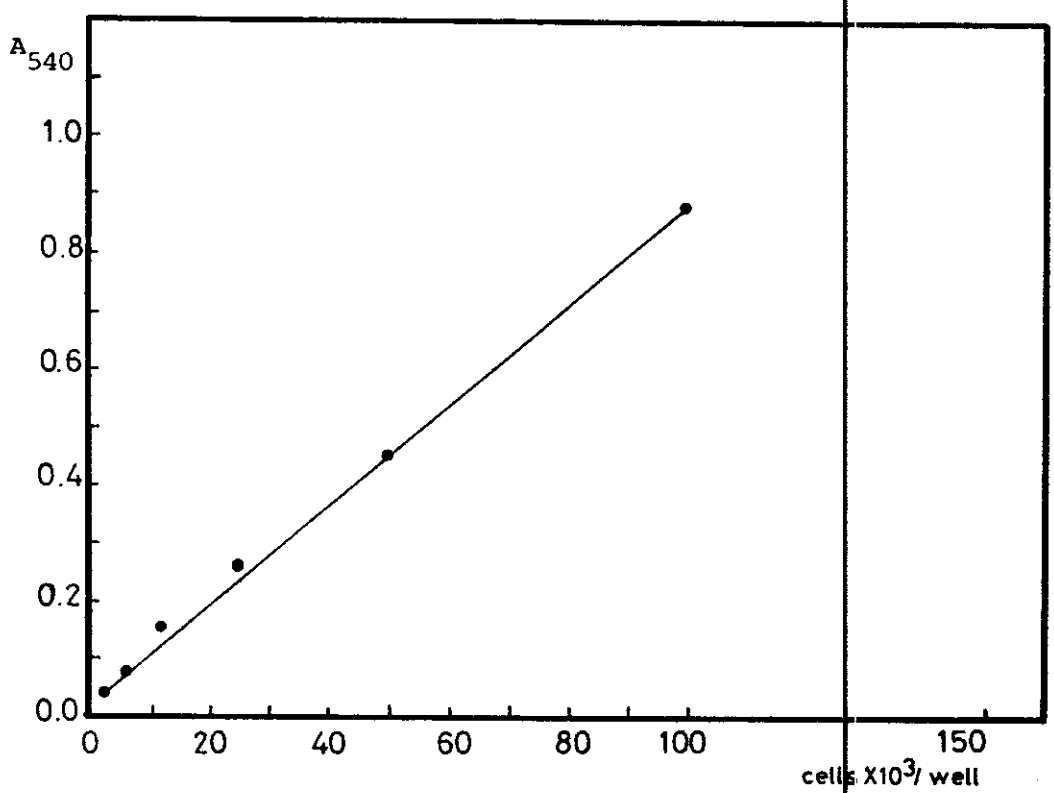


TABLE XI

SPECIFICITY OF THE MTT/MICROCOLORIMRTRIC LA[illegible]-ASSAY

Exp. Gp.	Extract	leukocytes	% adherence (Abs. ± SE)	Mean % adherence (Abs. ± SE)
A	none	Con	0.74 ± 0.05	0.77 ± 0.03
			0.80 ± 0.04	
			0.76 ± 0.02	
		SqCC/UB	0.81 ± 0.02	0.82 ± 0.04
			0.86 ± 0.04	
			o.79 ± 0.03	
B	SqCC/UB1/82	Con	0.71 ± 0.02	0.77 ± 0.2
			0.78 ± 0.04	
			0.83 ± 0.01	
		SqCC/UB	0.42 ± 0.01	0.44 ± 0.05 (P <0.01)
			0.39 ± 0.12	
			0.51 ± 0.02	

antigenic material and 0.71, 0.78 and 0.83 in presence of SqCC/UB1/82 extract. Peripheral blood mononuclear cells obtained from 3 SqCC/UB patients showed absorbances of 0.81, 0.86 and 0.79 in protein-free medium but of 0.42, 0.39 and 0.51 in the presence of the same extract. Mean % adherence is also illustrated in the table.

B: TPC/Microcolorimetric-LAI test

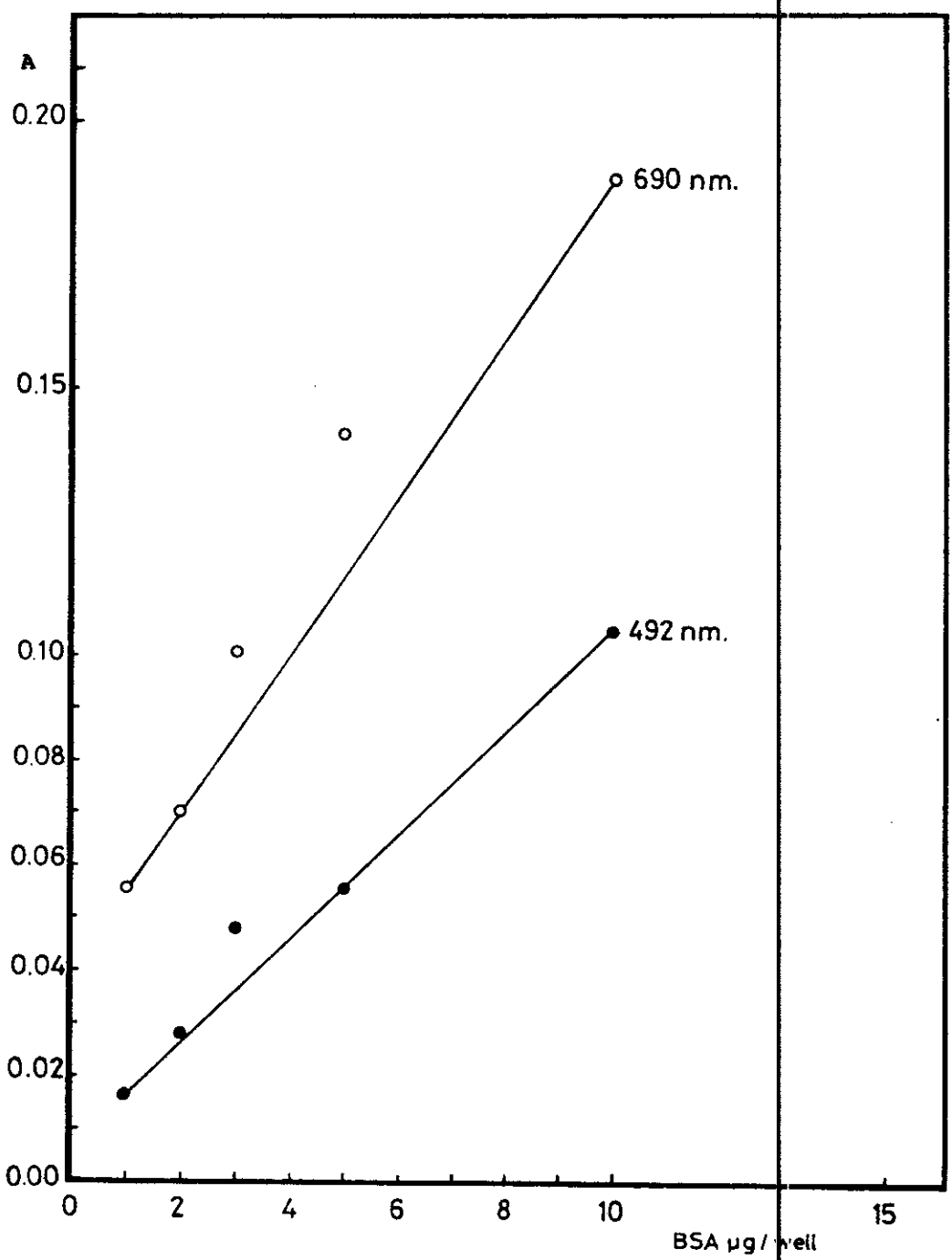
Since this technique comes as a colorimetric modification of the LAI-micro assay, it depends on the measurment of total protein content of all adherent cells per well using Lowry's method. The pilot experiment was that of determining the standard correlation between protein content per well (as crystalline BSA) and absorbances at 492 nm. As the usefull range of assay is from 0.005 to 0.2 mg protein, a suggested range for establishing the calibration curve from 0.01 to 0.1 mg was used. This range was corresponding to that from 1 to 15 ug/well. Figure V shows the determined standard curve with a positive correlation coofficient of 0.76 (76% correlation). Measurements of absorbances were also determined at 690 nm (a more sensitive wave length for concentrations of proteins less than 125 ug). The standard curve, also illustrated in Figure V, showed a positive correlation coofficient of 0.74 (74% correlation). The correlation showed by the data obtained at the 2 wave lengths (492 and 690 nm) were significantly identical with an increase in the sensitivity at 690 nm.

The 492 nm wave length channel on the multiscan spectrophotometer was used to determine the correlation between cell

FIGURE V

CORRELATION BETWEEN BSA CONCENTRATION AND

MEASURED ABSORBANCES



number and absorbances. Figure VI illustrates the standard correlation between PBMC count per well, in the range between $10x10^3$ and $100x10^3$ in a 10-fold increaments and corresponding absorbances measured at 492 nm. The calculated correlation coofficient (r) was 0.86 (data show 86% correlation).

The next step in establishing this colorimetric modification of the LAI microassay was to study the relation between the microscopic count of adherent PBMC per field in absence of any protein material (RPMI-1640 medium only) and corresponding absorbances measured at 492 nm. This relation is illustrated in Figure VII with a positive correlation of 67.8 % (r = 0.678). The actual cell count per well was in the range from $30x10^3$ to $100x10^3$ corresponding to visual count from 15 to 82 per microscopic field at 400x magnification. Table XII shows the relation between the 3 measured parameters, namely: PBMC count $x10^3$ per well, visual PBMC count per field and absorbances measured at 492 nm. It is quite observable that as the cultured PBMC per well increase, the count per field and the absorbance both increase. This is a suitable condition for monitoring the adherent cells by measuring the total protein content expressed as absorbances.

Now haw much this modification of the micro-LAI assay is capable of measuring a specific reactivity between an antigenic extract and the sensetized leukocytes? Table XIII shows the results of an experiment in which 3 groups of leukocytes have exposed to the modified colorimeric measurement of adherent cells in protein-free

FIGURE VI

CORRELATION BETWEEN PBMC COUNT AND MEASURED ABSORBANCE

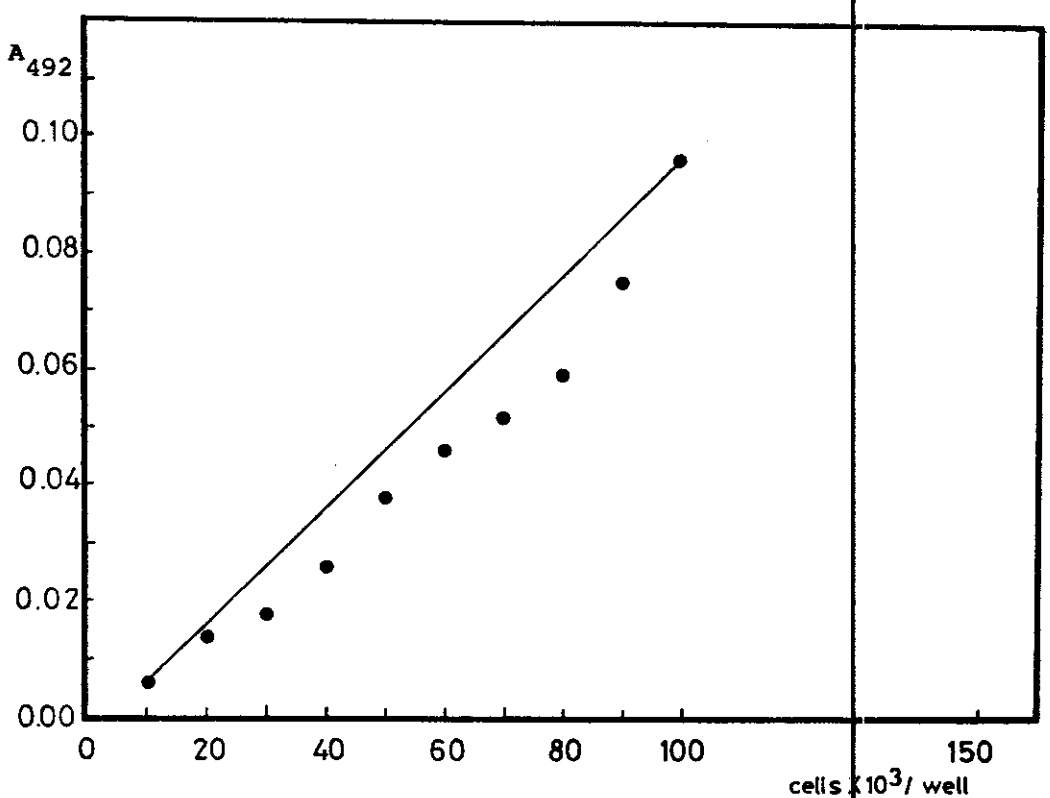


FIGURE VII

CORRELATION BETWEEN PBMC COUNT PER MICROSCOPIC FIELD AND MEASURED ABSORBANCE.

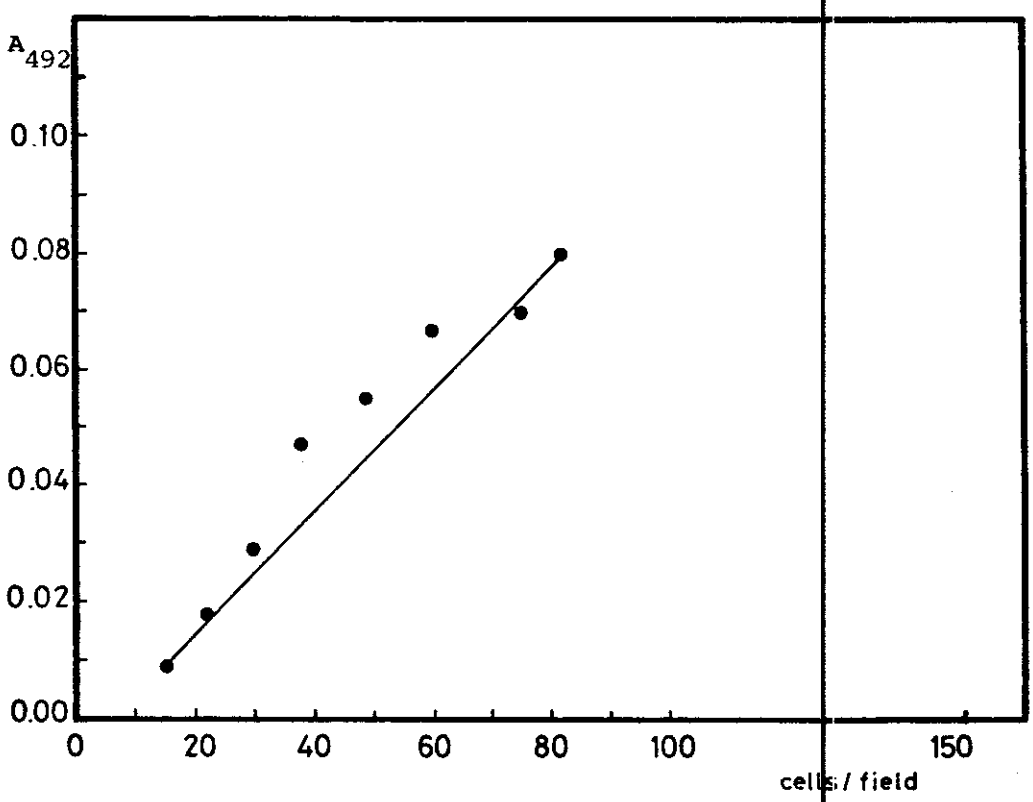


TABLE XII

THREE PARAMETER RELASIONSHIP OF THE TPC/MICROCOLORIMETRIC-LAI

Cultured PBMC X 10^3/well (mean ± SE)	PBMC count/field (mean ± SE)	Absorbance at 492 (mean ± SE)
35 ± 1.9	15 ± 0.7	0.009 ± 0.001
42 ± 0.9	22 ± 1.1	0.018 ± 0.001
50 ± 1.7	30 ± 1.1	0.029 ± 0.002
58 ± 1.7	38 ± 1.7	0.047 ± 0.002
69 ± 2.4	49 ± 1.3	0.055 ± 0.001
80 ± 1.8	60 ± 1.6	0.067 ± 0.001
95 ± 1.5	75 ± 1.8	0.070 ± 0.001
100 ± 1.4	82 ± 1.8	0.080 ± 0.002

TABLE XIII

SPECIFICITY OF THE TPC/MICROCOLOURIMETRIC LAI-ASSAY

Extract	Exp. Gp.	leukocytes	Adherent cells (Abs. ± SE)	Mean adherent cells (Mean Abs. ± SE)
None	A	Con	0.089 ± 0.025 0.090 ± 0.027 0.077 ± 0.025 0.074 ± 0.030 0.074 ± 0.030 0.071 ± 0.029 0.087 ± 0.028	0.080 ± 0.003
	B	Shaem	0.067 ± 0.005 0.071 ± 0.005 0.070 ± 0.005	0.071 ± 0.002
		SqCC/CX	0.079 ± 0.026 0.069 ± 0.030 0.080 ± 0.006	
		TrCC/UB	0.062 ± 0.005	
	C	SqCC/UB	0.071 ± 0.017 0.079 ± 0.027 0.069 ± 0.030 0.062 ± 0.005 0.067 ± 0.007 0.070 ± 0.005 0.087 ± 0.027	0.072 ± 0.003
SqCC/UB1/83	A	Con	0.073 ± 0.006 0.086 ± 0.023 0.069 ± 0.030 0.070 ± 0.005 0.080 ± 0.003 0.071 ± 0.005 0.066 ± 0.025	0.073 ± 0.003
	B	Shaem	0.079 ± 0.030 0.064 ± 0.007 0.067 ± 0.006	0.071 ± 0.002
		SqCC/CX	0.069 ± 0.005 0.078 ± 0.025 0.064 ± 0.030	
		TrCC/UB	0.075 ± 0.024	
	C	SqCC/UB	0.048 ± 0.023 0.049 ± 0.027 0.058 ± 0.025 0.052 ± 0.007 0.055 ± 0.005 0.060 ± 0.026 0.034 ± 0.030	0.051 ± 0.003 (P <0.05)

RPMI-1640 medium and in presence of the reactive SqCC/UB1/83 antigenic extract. PBMC of each groups A,B and C expressed levels of adherence in nearly the same level with means of 0.080 ± 0.003, 0.071 ± 0.002 and 0.072 ± 0.003. SqCC/UB sensitized PBMC samples showed a remarkably lower values of adherence (expressed as abdorbances) in presences of the SqCC/UB1/83 extract with a mean value of 0.051 ± 0.003. This value indicated high sensitivity of the TPC/micro-LAI test. The specificity of the test is quit clear when this value (0.051 ± 0.003) was compared with means of adherence of normal and control groups.

PART THREE: REACTION MECHANISM OF THE LAI ASSAY

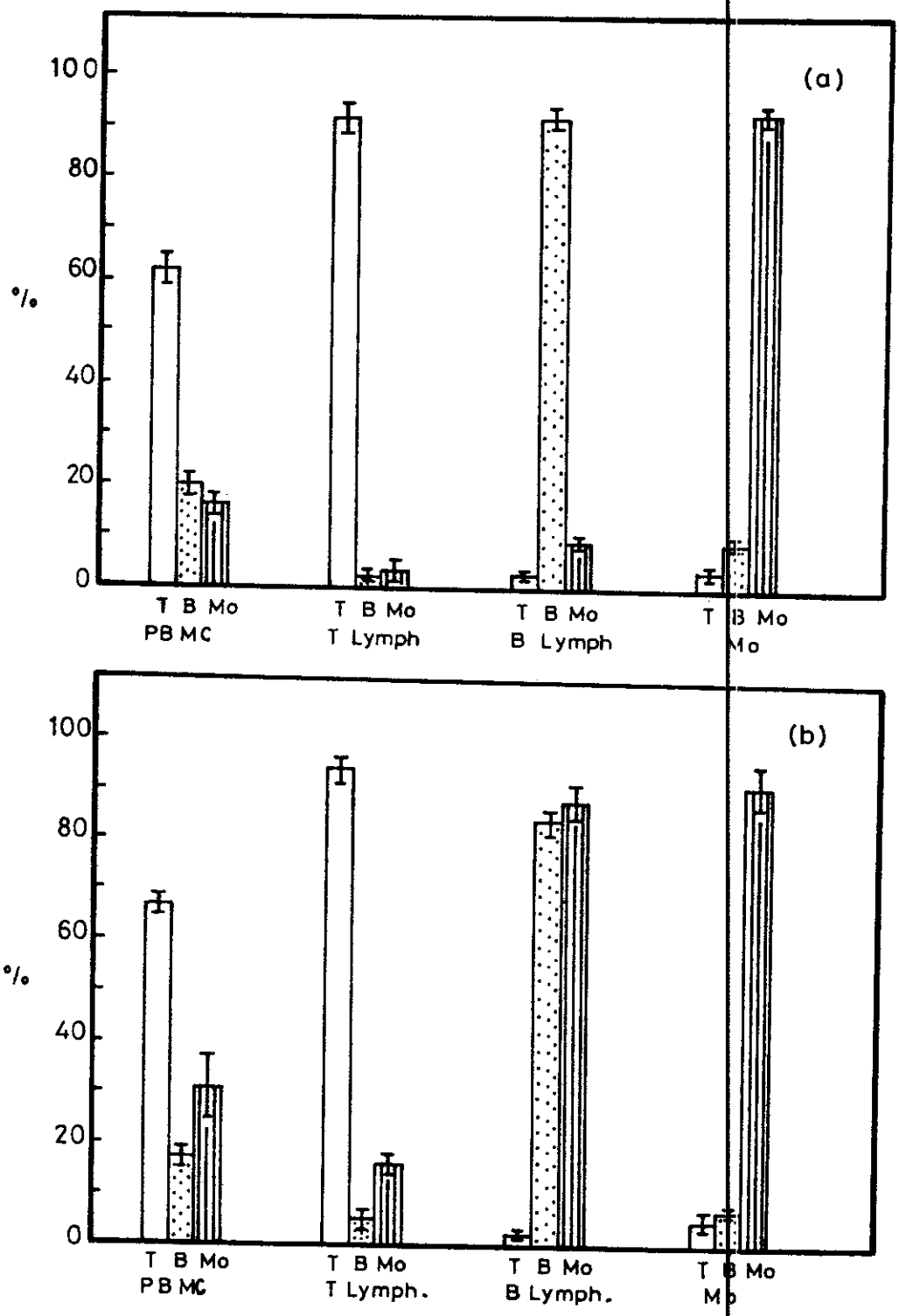
In studying the mechanism involved in the LAI reaction, and because of the variation in the theories according to variation in the LAI assays, different study experiments have been performed. These experiments were of four main types namely: experiments depending on isolation of leukocyte subpopulations (direct subpopulation experiments), experiments based on depletion of leukocyte subpopulation (depletion experiments), experiments detecting any secretions in culture supernatants (supernatant experiments) and mixed cell experiments (criss-cross experiments).

Before starting the above mentioned experiments the purity of PBMC in addition to that of isolated fractions (T, B cells or monocytes) were monitored. Figure VIIIa shows the percentages of the three lymphocyte subpopulations using traditional methods (EAET - rosetting, immunofluorescence and latex-ingestion techniques). PBMC were found to contain 62% T lymphocytes, 20% B lymphocytes and 16% monocytes. Isolated T lymphocyte subpopulation showed a purity of 92%, 2% contamination of B lymphocytes and 3% of monocytes. B lymphocyte subpopulation also showed the same level of 92% purity with 2% and 9% residual of T cells and monocytes, respectively. A 93% purity level was expressed by separated monocytes. Residual T and B cells were 3% and 9% in this preparation.

Using monoclonal antibodies, OKT3, OKB7 and OKIa1, in the

FIGURE VIII

MONITORING OF LYMPHOCYTE SUBPOPULATIONS PURITY



immunoperoxidase staining technique, PBMC expressed ratios of 67% T lymphocytes, 17% B lymphocytes and 31% B lymphocytes together with monocytes. By a simple substraction, monocytes were found to be of less than 14%. T lymphocyte and B lymphocyte-isolated subpopulations showed, respectively 94% and 84% levels of purity. Monocyte subpopulation showed more than 79% purity level (Figure VIIIb).

Conclusively both traditional methods and immunoperoxidase staining technique measured high levels of purity of cell subpopulations.

I: Direct Subpopulation Experiments

This kind of experiments aimed to compare between adherent cells (expressed as absorbance) of SqCC/UB patients with those obtained from control subjects. The comparison was done between not only PBMC adherence levels but also between the 3 subpopulations (T, B cells and monocytes) adherences when exposed to incubation with SqCC/UB1/82 antigenic extract. PBMC obtained from 5 control donors showed absorbance range from 0.069 to 0.088 while those delivered from 14 SqCC/UB patients expressed a range of absorbanc from 0.049 to 0.087. T lymphocyte subpopulations isolated from PBMC of the same controle donors ranged in their adherence from 0.048 to 0.076. The same subpopulation of SqCC/UB patients expressed values of adherence between 0.036 and 0.083. Range of adherence of B lymphocyte subpopulations of control donors was from 0.059 to 0.081 and that of SqCC/UB patients was from 0.038 to 0.086. The monocyte subpopulation

TABLE IXV

EFFECT OF SqCC/UB1/83 EXTRACT ON LEUKOCYTE ADHERENCE

Leukocytes	Adherent cells (Abs.± SE)			
	PBMC	T cells	B cells	Monocytes
Con	0.071 ± 0.003	0.043 ± 0.007	0.064 ± 0.003	0.063 ± 0.002
	0.088 ± 0.002	0.073 ± 0.003	0.059 ± 0.003	0.071 ± 0.003
	0.083 ± 0.005	0.074 ± 0.003	0.061 ± 0.002	0.082 ± 0.004
	0.081 ± 0.005	0.076 ± 0.004	0.081 ± 0.006	0.088 ± 0.004
	0.059 ± 0.003	0.073 ± 0.005	0.072 ± 0.002	0.067 ± 0.003
Mean	0.078 ± 0.004	0.068 ± 0.025	0.067 ± 0.030	0.074 ± 0.033
SqCC/UB	0.052 ± 0.007	0.039 ± 0.007	0.066 ± 0.004	0.039 ± 0.025
	0.049 ± 0.003	0.038 ± 0.008	0.051 ± 0.025	0.021 ± 0.025
	0.079 ± 0.003	0.072 ± 0.003	0.069 ± 0.004	0.013 ± 0.030
	0.080 ± 0.003	0.083 ± 0.003	0.053 ± 0.004	0.029 ± 0.020
	0.061 ± 0.006	0.042 ± 0.006	0.040 ± 0.003	0.031 ± 0.004
	0.060 ± 0.006	0.037 ± 0.006	0.043 ± 0.004	0.042 ± 0.002
	0.087 ± 0.003	0.049 ± 0.006	0.047 ± 0.004	0.022 ± 0.007
	0.068 ± 0.007	0.042 ± 0.008	0.038 ± 0.004	0.037 ± 0.006
	0.071 ± 0.002	0.039 ± 0.008	0.070 ± 0.003	0.041 ± 0.006
	0.061 ± 0.003	0.036 ± 0.001	0.061 ± 0.006	0.017 ± 0.006
	0.060 ± 0.003	0.043 ± 0.002	0.073 ± 0.003	0.013 ± 0.006
	0.063 ± 0.003	0.047 ± 0.002	0.039 ± 0.008	0.028 ± 0.003
	0.057 ± 0.004	0.036 ± 0.007	0.081 ± 0.003	0.033 ± 0.004
	0.082 ± 0.002	0.053 ± 0.006	0.086 ± 0.003	0.042 ± 0.006
Mean	0.066 ± 0.003 (P <0.05)	0.047 ± 0.004 (P <0.05)	0.058 ± 0.004 (P <0.05)	0.029 ± 0.003 (P <0.01)

isolated from the same group of control donors expressed a range of adherence between 0.063 and 0.088. On the other hand SqCC/UB patients monocyte subpopulations showed a range of adherence between 0.013 and 0.042. These results, together with their mean values were shown in Table IXV. PBMC, T cells, B cells and monocyte subpopulations showed a mean adherences of 0.078 ± 0.004, 0.068 ± 0.025, 0.067 ± 0.030 and 0.074 ± 0.033 respectively. Corresponding values obtained with SqCC/UB patients' samples were 0.066 ± 0.003, 0.047 ± 0.004, 0.058 ± 0.004 and 0.029 ± 0.003. By comparing these mean values it became obvious that a specific adherence inhibition was expressed by PBMC, T cells, B cells and monocytes of sensitized patients. It is worth mentioning the lower adherence inhibition elicited by T cells from SqCC/UB samples.

II: Depletion Experiments

With the PBMC subpopulation depletion experiments we can observe the effect of missing cell-type and the effect of the remaining double cell population on the adherence level in presence of the chosen reactive SqCC/UB1/82 extract (Table XV). Fifteen blood samples derived from 5 control donors and 10 SqCC/UB patients were tested. PBMC in addition to T+B, T+Mo and B+Mo double-cell subpopulations were the test groups of all previously mentioned samples. Mean adherence leveles of the 4 cell groups obtained from the controle donors were 0.073 ± 0.004, 0.060 ± 0.005, 0.066 ± 0.005 and 0.072 ± 0.006. The same 4 cell groups isolated from blood samples of the SqCC/UB patients expressed mean values of adherence of 0.055 ± 0.001,

TABLE XV

EFFECT OF LEUKOCYTE SUBPOPULATION DEPLETION ON SPECIFIC ADHERENCE REDUCTION

Leukocytes	Adherent cells (Abs. ± SE)			
	PBMC	T+B Cells	T+Mo cells	B+Mo cells
Con	0.078 ± 0.006	0.069 ± 0.025	0.061 ± 0.001	0.065 ± 0.002
	0.079 ± 0.006	0.071 ± 0.025	0.087 ± 0.007	0.066 ± 0.002
	0.069 ± 0.002	0.042 ± 0.002	0.065 ± 0.003	0.058 ± 0.002
	0.058 ± 0.002	0.064 ± 0.025	0.062 ± 0.003	0.087 ± 0.002
	0.082 ± 0.002	0.053 ± 0.002	0.055 ± 0.003	0.085 ± 0.001
Mean	0.073 ± 0.004	0.060 ± 0.005	0.066 ± 0.005	0.072 ± 0.006
SqCC/UB	0.067 ± 0.002	0.045 ± 0.008	0.054 ± 0.003	0.045 ± 0.007
	0.051 ± 0.002	0.043 ± 0.006	0.051 ± 0.003	0.055 ± 0.007
	0.062 ± 0.002	0.066 ± 0.006	0.060 ± 0.020	0.047 ± 0.002
	0.062 ± 0.010	0.067 ± 0.003	0.071 ± 0.001	0.031 ± 0.021
	0.040 ± 0.008	0.059 ± 0.006	0.039 ± 0.030	0.025 ± 0.030
	0.059 ± 0.004	0.079 ± 0.002	0.072 ± 0.001	0.033 ± 0.030
	0.062 ± 0.002	0.040 ± 0.001	0.052 ± 0.004	0.043 ± 0.006
	0.060 ± 0.004	0.038 ± 0.001	0.050 ± 0.003	0.037 ± 0.002
	0.049 ± 0.004	0.076 ± 0.002	0.043 ± 0.003	0.044 ± 0.001
	0.034 ± 0.007	0.043 ± 0.006	0.038 ± 0.002	0.055 ± 0.001
Mean	0.055 ± 0.001 ($P < 0.05$)	0.056 ± 0.005 ($P < 0.05$)	0.053 ± 0.004 ($P < 0.05$)	0.042 ± 0.003 ($P < 0.01$)

0.051 ± 0.005, 0.053 ± 0.004 and 0.042 ± 0.003 respectively. The highest level of adherence reduction is obviously in the B+Mo population of sensitized origen.

III: Supernatant Experiments

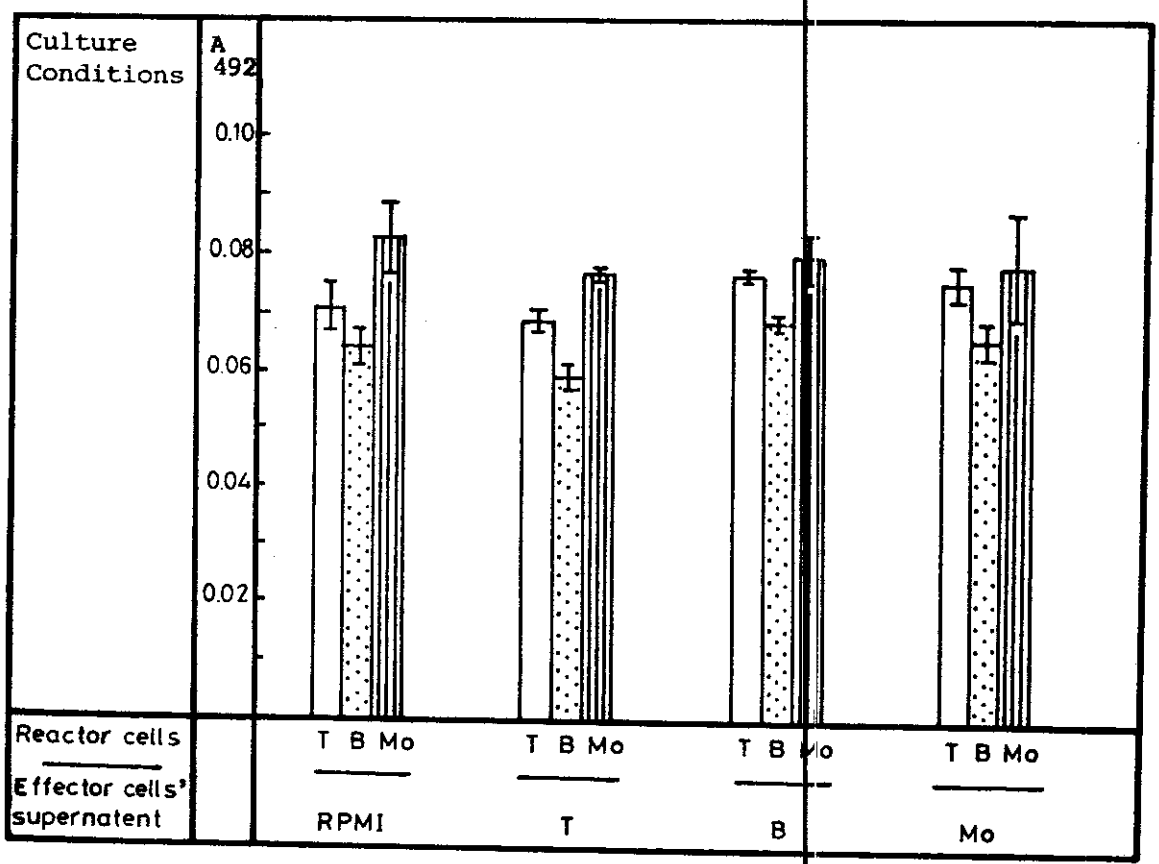
Three subpopulations (T, B cells and monocytes) were isolated from 2 blood samples of SqCC/UB patients (effector cells) were cultured with the SqCC/UB1/82 extract for 2 hours. The culture supernatants collected by centrifugation were used in LAI assay cultures instead of the antigenic extract together with the same 3 subpopulations obtained from 1 control donor (reactor cells). When the reactor cell subpopulations were cultured in absence of any extract, in protein-free RPMI-1640 medium (Figure IX) mean levels of adherence of the 3 PBMC subpopulations were 0.071 ± 0.004, 0.064 ± 0.003 and 0.083 ± 0.006, respectively. No difference was observed in adherence of PBMC subpopulations (reactor cells) when exposed to LAI assay in presence of supernatants collected from cultures of T, B cells or monocytes (effector cells) in presence of the SqCC/UB1/82 extract (Figure IX). These results indicate the secretion of no mediator by sensitized T cells or any other subpopulation.

IV: Criss-Cross Experiments

PBMC obtained from 2 patients with squamous cell carcinoma of urinary bladder and from 1 control donor were subpurified into the T, B cells and monocyte subpopulations. Each of the separated

FIGURE IX

EFFECT OF CULTURE SUPERNATENT ON THE LEUKOCYTE SUBPOPULATION ADHERENCE.



cell types was mixed with the 3 types of populations of the nonsensitized leukocyte subsets in 1:1 ratio. The mixed cells were tested by the TPC/Micro-LAI modified technique both in absence and presence of the reactive SqCC/UB1/82 antigenic extract. Individual results are plotted in Table XVI. Sensitized monocytes of the 2 patients greatly reduced the adherence of normal T cells and monocytes (more than 20% adherence reduction). Sensitized monocytes of one patient reduced to the same level the adherence of nonsensitized B cells but the other did not.

B cells of the two patients lowered the adherence of the control monocytes in different levels (42.9% and 17.9% adherence reduction). T lymphocytes of the first patient reduced the adherence of control monocytes to the level of 11.1%, but these of the second patient only reduced the adherence of the normal B cells to the level of 11.5%.

TABLE XVI

EFFECT OF MIXED SUBPOPULATION CULTURES

Extract	Leukocytes Sensitized	/ Nonsensitized			Adherent cells (Abs. ± SE)	Adherence* reduction %
None	SqCC/UB	T_1	Con	T	0.073 ± 0.007	
				B	0.068 ± 0.005	
				Mo	0.081 ± 0.008	
		B_1	Con	T	0.069 ± 0.009	
				B	0.063 ± 0.009	
				Mo	0.084 ± 0.007	
		Mo_1	Con	T	0.091 ± 0.009	
				B	0.069 ± 0.007	
				Mo	0.088 ± 0.006	
	SqCC/UB	T_2	Con	T	0.069 ± 0.008	
				B	0.078 ± 0.006	
				Mo	0.071 ± 0.009	
		B_2		T	0.070 ± 0.008	
				B	0.065 ± 0.007	
				Mo	0.078 ± 0.006	
		Mo_2		T	0.080 ± 0.007	
				B	0.072 ± 0.003	
				Mo	0.089 ± 0.006	
SqCC/UB	SqCC/UB	T_1	Con	T	0.080 ± 0.008	----
				B	0.068 ± 0.003	----
				Mo	0.072 ± 0.007	11.1
		B_1		T	0.074 ± 0.003	----
				B	0.067 ± 0.007	----
				Mo	0.078 ± 0.010	42.9
		Mo_1		T	0.041 ± 0.030	54.9
				B	0.043 ± 0.009	37.7
				Mo	0.020 ± 0.010	77.3
	SqCC/UB	T_2		T	0.074 ± 0.003	----
				B	0.069 ± 0.010	11.5
				Mo	0.077 ± 0.020	----
		B_2		T	0.078 ± 0.002	----
				B	0.068 ± 0.010	----
				Mo	0.064 ± 0.009	17.9
		Mo_2		T	0.073 ± 0.002	8.8
				B	0.068 ± 0.010	----
				Mo	0.013 ± 0.020	85.4

* Adherence reduction =

$$\left[\frac{\text{Abs. in absence of extract} - \text{Abs. in presence of extract}}{\text{Abs. in absence of extract}}\right] \times 100$$