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SIGNIFICANCE OF SERUM FACTORS MODIFYING CELLULAR IMMUNE RESPONSES TO GROWING TUMOURS

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Summary.—Lymphocytes from hepatoma bearing rats are cytotoxic for cells of the corresponding hepatoma and this reactivity can be specifically blocked by pretreating target cells with tumour bearer serum. This blocking activity is unlikely to be mediated by circulating tumour specific antibody, since none is detectable by cytotoxic assay in these sera. In contrast, serum from tumour immune rats is cytotoxic for plated hepatoma cells, but in the absence of complement these sera block lymphocyte cytotoxicity, findings which suggest that blocking by humoral antibody may not be a significant factor in modifying tumour growth. It has also been established that immune complexes prepared by adding hepatoma immune serum to solubilized tumour specific antigen can block target hepatoma cells from lymphocyte attack and this is probably the mechanism of blocking by tumour bearer serum. More significantly, lymph node cell cytotoxicity is specifically inhibited following incubation with tumour bearer serum. Similar lymphocyte inhibition is obtained by pretreating effector cells with solubilized hepatoma specific antigen. This suggests that the activity of tumour bearer serum may be effected by circulating tumour antigen or immune complexes and in support of this, there is evidence for these factors in the sera studied. These investigations indicate that inhibition of lymphocyte reactivity by tumour bearer serum is of a complex nature, but probably the most relevant with regard to *in vivo* immune responses is the tumour-antigen mediated inhibition of lymphocyte reactivity.

STUDIES with experimental and human tumours indicate that lymphocytes from the tumour bearing host are specifically sensitized to cell surface expressed tumour associated antigens and are cytotoxic *in vitro* for cultured tumour cells (reviewed by Hellström and Hellström, 1969*b*, 1970*b*). These findings led to the proposal that the serum of tumour bearing individuals may contain factors which interfere with the effective mediation of cellular immunity resulting in, or contributing to, a failure of immunological control of tumour growth (Hellström and Hellström, 1969*a*) and, initially, this effect was ascribed to " blocking antibody ". In support of this concept, there is now considerable evidence in a number of tumour systems, both in experimental animals and cancer patients, indicating that preexposure of cultured tumour cells to serum from tumour bearers reduces their susceptibility to attack by specifically sensitized lymphocytes (Hellström and Hellström, 1969*a*; Hellström, Hellström and Sjögren, 1970; Ankerst, 1971; Baldwin, Embleton and Robins, 1973*b*; Bubeník *et al.*, 1970; Hellström *et al.*, 1971*b*). More recent evidence, however, suggests that antibody alone may not be of primary importance in blocking tumour cells from lymphocyte attack, this effect being mediated by immune complexes (Sjögren *et al.*, 1971; Baldwin, Price and Robins, 1972).

The objective of the present studies, using aminoazo dye-induced rat hepatomata, was to analyse comprehensively the nature and site of interaction of humoral factors from tumour bearers which inhibit the cytotoxicity of sensitized lymphocytes for cultured rat hepatoma cells. In this context, interactions between humoral factors and target tumour

cells are referred to as blocking effects, whereas interactions with lymphocytes leading to desensitization are termed inhibitory reactions.

MATERIAL AND METHODS

Rats and tumours.—Hepatomata were induced in Wistar rats following oral administration of 4-dimethylaminoazobenzene and were maintained by serial subcutaneous passage in syngeneic recipients (Baldwin and Barker, 1967*a*).

Tumour immunization.—Syngeneic rats were immunized against transplanted hepatomata by implantation of γ-irradiated tumour tissue as previously described (Baldwin and Barker, 1967*a*). Immunized rats rejected challenges of 5×10^5 tumour cells which grew consistently in untreated controls.

Lymphocyte cytotoxicity assays.—Monolayer cell lines established from primary and transplanted hepatomata were used as source of target cells for colony inhibition and microcytotoxicity assays, which were performed as previously described (Baldwin and Embleton, 1971; Baldwin *et al.*, 1973*b*; Baldwin, Price and Robins, 1973*d*).

Serum blocking of lymph node cell cytotoxicity.—Hepatoma cells were plated in the wells of Microtest plates (Falcon No. 3040 or Cooke M29 ART) and after incubation to allow cell adhesion, medium was replaced by test or control serum. After 45 minutes incubation at 37°C, serum was removed and the susceptibility of the treated tumour cells to lymph node cells from hepatoma immune rats assayed (Baldwin *et al.*, 1973*b*, *d*).

Inhibition of lymph node cell cytotoxicity.—Lymph node cell suspensions were incubated for 45–60 minutes at 37°C with reactant (serum or papain-solubilized hepatoma D23 antigen). The cells were then recovered by centrifugation (120 *g* for 5 minutes), resuspended in Eagle's MEM medium and their cytotoxicity determined.

RESULTS

Immune reactions in the hepatoma bearing host

The results of colony inhibition tests with lymph node cells from hepatoma bearing rats are shown in Table I. Significant lymphocytotoxicity for cultured cells of the autologous hepatoma was shown by 13 of the 17 lymph node cell preparations from transplanted hepatoma bearing rats. Comparably, all 3 lymph node cell preparations from primary hepatoma-bearers were significantly cytotoxic for these tumours. In these experiments, target hepatoma cells were established in culture from tumour tissue specimens taken at biopsy, thus allowing lymph node cells to be tested against autochthonous target cells. In tests with both primary and transplanted hepatomata, the individual specificity of the rejection antigens was reflected so that, for example, lymph

TABLE I.—*Cytotoxicity of Lymph Node Cells (LNC) from Rats Bearing Primary and Transplanted Hepatomata*

Target hepatoma	Lymph node cell donor	Percentage cytotoxicity†
D23	D23	27·8***, −3·5, −7·9, 24·9***, 23·6**, 22·3**, 32·9**
D30	D30	5·2, 25·0*, 33·7***, 8·6, 26·8***
D30	D31	5·7
	D23	10·8
D31	D31	22·0**, 29·5**, 9·8*, 33·6***, 27·7**
D185	D185‡	36·3**
D187	D187‡	37·2***
D189	D189‡	33·1***
	D193‡	−2·2

† Determined by inhibition of colony formation in comparison with lymph node cells from normal rats (Baldwin and Embleton, 1971).

‡ Lymph node cells taken from primary hepatoma bearing rat.

* $P < 0{\cdot}05$. ** $P < 0{\cdot}005$. *** $P < 0{\cdot}0005$.

node cells from a rat bearing hepatoma D30 were cytotoxic for D30 target cells (26·8% cytotoxicity) but not for D31 target cells (5·7% cytotoxicity).

Humoral factors interfering with lymphocyte cytotoxicity for tumour cells

Serum from hepatoma bearing rats was tested to determine whether circulating factors capable of interfering with cellular immunity reactions *in vitro*, accompany progressive tumour growth. Plated hepatoma target cells were incubated with heat inactivated sera for 45–60 minutes, then the sera were removed and the susceptibility of the treated target cells to the cytotoxic effect of lymph node cells from hepatoma immune rats was determined. As exemplified by data shown in Table II, pretreatment of hepatoma target cells with tumour bearer serum blocked specifically the cytotoxic effect of hepatoma immune lymph node cells. The tumour specificity of this effect is illustrated in Experiments 2 and 4 where pretreatment of hepatoma cells with serum from rats bearing an antigenically different hepatoma did not block lymphocyte cytotoxicity.

These observations are compatible with results obtained in other studies using experimental tumours induced by oncogenic viruses and chemical carcinogens (Hellström and Hellström, 1970*b*; Ankerst, 1971). There is also clear evidence from investigations with several types of human neoplasm that serum from tumour bearing patients can specifically block cells of the appropriate tumour type from attack by cytotoxic lymphocytes (Bubeník *et al.*, 1970; Hellström *et al.*, 1971*b*).

Nature of serum blocking factors

The concept that antibody is involved in blocking tumours cells from lymphocyte attack is supported by the finding that the blocking factor in the serum of mice bearing progressively growing Moloney virus induced sarcomata could be neutralized by the addition of goat anti-mouse 7*s* immunoglobulin (Hellström and Hellström, 1969*a*). This blocking factor could also be removed by absorbing tumour bearer serum with intact tumour cells and preliminary serum fractionation by Sephadex G-200 gel filtration indicated that it was separated in the 7*s* fraction.

Fractionation studies have demonstrated that the blocking activity of serum from rats bearing hepatoma D23 is also associated with 7*s* immunoglobulins. Initially, serum proteins were separated into a series of fractions with different molecular weight ranges by zone centrifugation through sucrose gradients. Diluted tumour bearer serum (0·5 ml of 1/2 dilution) was layered onto 25 ml 6% to 20% linear sucrose gradients. After cen-

TABLE II.—*Blocking of Lymph Node Cell (LNC) Cytotoxicity with Serum from Rats Bearing Primary and Transplanted Hepatomata*

Experiment	Target hepatoma	Serum donor	Colony numbers. (Mean ±S.E.) Normal LNC	Immune LNC*	Percentage cytotoxicity	Percentage blocking
1	D23	Normal	186·0±4·9	130·0±3·0	30·0	
		D23 tumour bearer	168·0±6·7	166·3±1·4	0·9	96·7
2	D23	Normal	150·7±8·7	115·0±13·6	23·7	
		D23 tumour bearer	112·3±0·7	123·7±14·0	−10·9	100
		D30 tumour bearer	122·3±16·2	77·7±16·7	36·5	0
3	D30	Normal	130·0±2·5	89·0±3·5	31·5	
		D30 tumour bearer	112·7±4·3	118·3±1·8	−5·0	100
4	D175	Normal	25·0±0·5	22·3±2·6	10·6	
		D175 tumour bearer†	21·0±1·0	25·3±0·8	−20·0	100
		D166 tumour bearer†	22·3±0·3	15·0±1·7	32·8	0

* Lymph node cells taken from rats immunized by implantation of irradiated hepatoma grafts and immune to challenge with 5×10^5 viable hepatoma cells.

† Serum taken from primary hepatoma bearing rat.

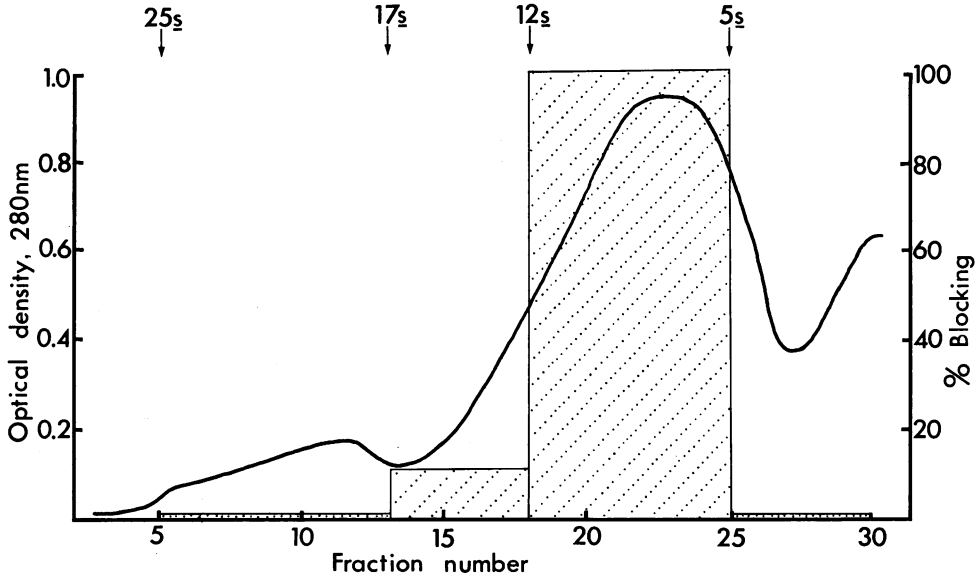

FIG. 1.—Blocking of lymphocyte cytotoxicity by fractions from hepatoma D23 bearer serum separated by zone centrifugation on linear sucrose gradients. Plated hepatoma D23 cells were exposed to fractions from hepatoma D23 bearer or, in controls, normal serum for 60 minutes. Fractions were then removed and the susceptibility of the tumour cells to immune lymph node cell cytotoxicity determined. Sedimentation coefficients for fraction boundaries were calculated by reference to standard proteins.

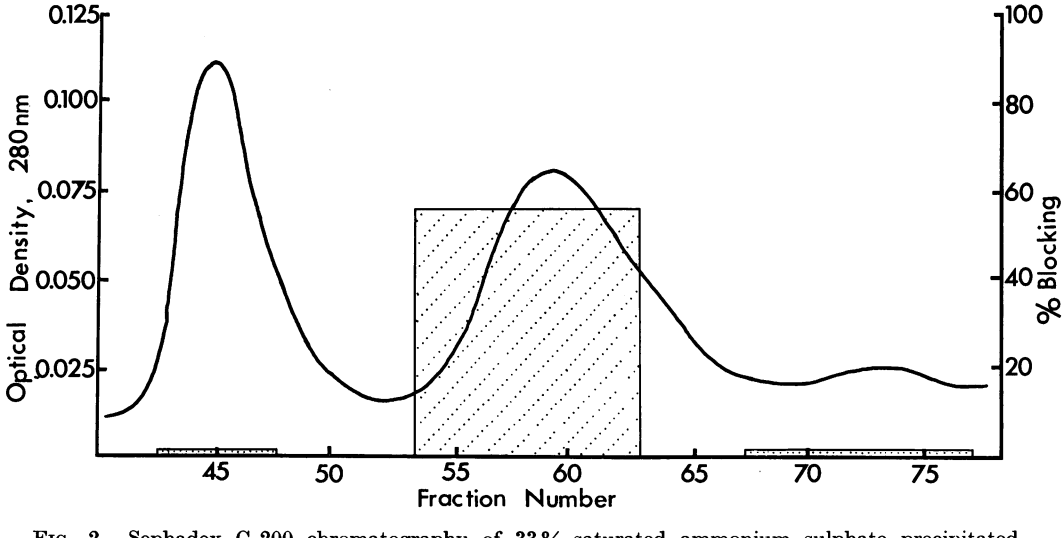

FIG. 2.—Sephadex G-200 chromatography of 33% saturated ammonium sulphate precipitated globulins from hepatoma D23 bearer serum in phosphate buffered saline, pH 7·3. The hatched area shows the location of blocking activity in the "7*s* zone" as determined by the capacity of fractions to protect plated hepatoma D23 cells from cytotoxic lymph node cells from hepatoma D23 immune rats.

trifugation at 25,000 rev/min in a Beckmann S.W.25.1 rotor for 16 hours, the gradient was collected from the bottom of the tube, and a typical elution profile is shown in Fig. 1. In this experiment, 4 pooled fractions were tested for blocking activity. One fraction located in the sedimentation coefficient range 5*s* to 12*s* exhibited significant blocking activity, in comparison with an equivalent fraction from normal rat serum so that hepatoma D23 cells treated with this fraction were completely protected from sensitized lymph node cells from tumour immune rats.

Serum from rats bearing transplants of hepatoma D23 was also fractionated by Sephadex G-200 chromatography (Fig. 2). A serum globulin fraction was initially precipitated with ammonium sulphate at 33% saturation and the precipitate redissolved in phosphate buffered saline pH 7·3 and dialysed against this solution before application to the G-200 column. The 19*s* and 4*s* fractions did not display any blocking, but the broad fraction containing 7*s* immunoglobulins exhibited significant activity (54·5% blocking). As in the zone centrifugation experiments, equivalent fractions from normal rat serum were used as controls in tests for blocking activity.

Although these studies broadly associate the blocking activity of hepatoma bearer serum with the 7*s* immunoglobulin fractions, several pieces of evidence are difficult to interpret on the basis of antibody alone as the blocking factor.

(a) Blocking sera from rats bearing primary and transplanted hepatomata generally do not contain antibody detectable by complement dependent cytotoxicity tests, although sera taken from rats following excision of hepatoma transplants, which lack blocking activity (*vide infra*), do have demonstrable cytotoxic antibody (Table III).

(b) A rapid loss of blocking activity after tumour excision or regression has been observed in several tumour systems. In a vertical study of serum blocking activity after excision of transplants of hepatoma D23, it was found that by 3–4 days post excision, sera no longer protected plated hepatoma D23 cells from immune lymphocyte cytotoxicity (Baldwin *et al.*, 1973*b*). In order to interpret these findings on the basis of antibody alone effecting blocking by tumour bearer serum, it is necessary to propose that the half-life of the antibody involved is very short to account for the rapid loss of blocking activity after tumour excision. In this case, a further difficulty is that, as indicated above, the loss of " blocking antibody " activity after excision is accompanied by the appearance of complement dependent cytotoxic antibody.

(c) In several studies it has also been reported that addition of serum from tumour free individuals abrogates (" unblocks ") the blocking activity of tumour bearer serum. This phenomenon can be demonstrated in the rat hepatoma system, using serum from rats taken after the excision of hepatoma transplants as the " unblocking " serum. As shown in Table IV, mixtures of equal volumes of tumour-bearer and post-excision serum lack blocking activity, although the blocking activity of tumour bearer serum is not overcome by smaller amounts of post-excision serum. These results are similar to those obtained with Moloney sarcomata in mice, where " regressor " serum abrogates the blocking activity of serum from mice bearing progressively growing tumours (Hellström and Hellström, 1970*a*). Serum from clinically symptom-free patients has also been shown to be " unblocking " (Hellström *et al.*, 1971*a*).

Blocking of lymphocyte cytotoxicity

An alternative proposal is that the blocking of sensitized lymphocyte cytotoxicity is mediated by antigen–antibody complexes. The complexes would not be expected to mediate complement-dependent cytotoxic reactions, and loss of serum blocking activity after tumour excision would reflect the rapid elimination of

TABLE III.—*Cytotoxicity Tests with Hepatoma Bearer and Post-excision Serum*

Target hepatoma	Serum donor	Percentage cytotoxicity†
D23	D23 tumour bearer	−33·7, 4·0, 4·1, 4·7, 19·9*, 1·2, 10·0
D23	D23 post excision	26·2**, 40·0***, 33·6***, 16·4**
D30	D30 tumour bearer	−6·4, −14·3
D30	D30 post excision	47·8**, 25·2*
D159	D159 tumour bearer‡	9·7
D164	D164 tumour bearer‡	0·0

† Determined by inhibition of colony formation, in comparison with serum from normal rats (Baldwin and Embleton, 1971).
‡ Serum taken from primary hepatoma bearing rat.
* $P < 0.05$. ** $P < 0.005$. *** $P < 0.0005$.

TABLE IV.—*Effect of Serum Taken after Excision of Hepatoma D23 on Blocking of Lymph Node Cell (LNC) Cytotoxicity by D23 Tumour Bearer Serum*

Experiment	Serum mixture	Volume ratio	Percentage cytotoxicity	Percentage blocking
1	Normal+P.E.*	1 : 1	25·4	
	T.B.†+Normal	1 : 1	−8·9	100
	T.B.+P.E.	1 : 1	40·9	0
2	Normal	—	32·6	—
	T.B.+Normal	1 : 1	5·5	83·2
	P.E.+Normal	1 : 1	36·1	0
	T.B.+P.E.	1 : 1	24·6	24·6
	T.B.+P.E.	2 : 1	2·4	92·6
	T.B.+P.E.	4 : 1	−0·4	100
3	Normal	—	35·2	—
	T.B.+Normal	1 : 1	−13·0	100
	T.B.+P.E.	1 : 1	14·3	59·3
	T.B.+P.E.	1 : 2	6·1	82·5

* P.E. Post-excision serum obtained 10 days after surgical excision of established hepatoma D23 grafts.
† T.B. Serum from rats bearing grafts of hepatoma D23.

immune complexes after removal of the antigen source, *i.e.* the tumour. The unblocking phenomenon could also be interpreted on the basis of neutralization of free antigenic determinants of the immune complexes which presumably are necessary for blocking activity.

Direct evidence that antigen–antibody complexes are capable of mediating blocking activity has been provided in model studies using transplanted rat hepatoma D23 (Baldwin *et al.*, 1972). The source of tumour specific antibody used in these studies was serum taken after the excision of hepatoma transplants. This serum lacks blocking activity, and contains antibody cytotoxic in the presence of complement. Hepatoma D23 has been studied extensively as a model system for the isolation and purification of plasma membrane expressed tumour antigens (Baldwin and Glaves, 1972; Baldwin, Harris and Price, 1973*c*). It has therefore been possible to prepare a solubilized tumour antigen by limited papain digestion of extranuclear membranes, which after DEAE cellulose chromatography yields a discrete antigenic fraction. In these studies, antigen has been characterized by its capacity to absorb specific antibody from hepatoma D23 immune serum, assayed by loss of membrane immunofluorescence reaction of absorbed sera with viable hepatoma cells in suspension.

Tumour specific antibody in the form of post excision serum, and papain-solubilized tumour antigen were incubated together, and the mixtures tested for blocking activity. As shown in Fig. 3, addition of 0·4 mg antigen preparation per

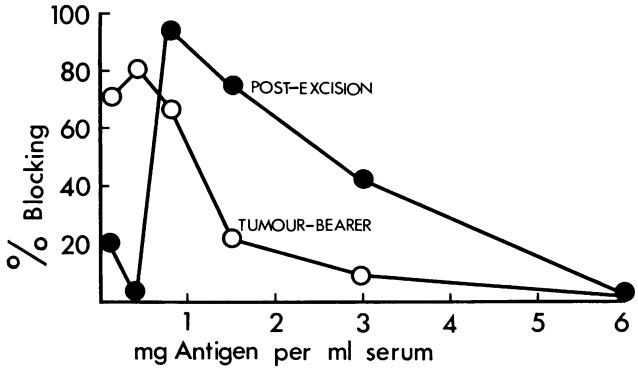

FIG. 3.—Modification of serum blocking activity following addition of papain-solubilized hepatoma D23 antigen. Post-excision serum was taken 14 days after excision of hepatoma D23 grafts. This serum contains cytotoxic antibody for hepatoma D23 cells, but shows no significant blocking activity. Following addition of appropriate amounts of solubilized hepatoma D23 antigen, blocking activity becomes detectable but this activity is lost in the region of excess antigen. Comparably, serum from tumour bearing rats initially shows a high blocking activity which is again lost when excess solubilized hepatoma D23 antigen is added. In these tests, hepatoma D23 cells were treated with serum or serum–antigen mixtures and the reactants removed before assaying their susceptibility to lymphocyte cytotoxicity.

ml post-excision serum did not result in blocking, although 0·8 mg antigen per ml was strongly blocking. As the proportion of antigen added was increased, the blocking activity was lost. The figure also shows that addition of the same antigen preparation to D23 tumour bearer serum abolished its blocking activity. It should be emphasized that in all these experiments, serum antigen mixtures were incubated with the target cells for 45–60 minutes and removed before addition of lymph node cells. In regions of antigen excess, therefore, free antibody sites would be occupied by solubilized antigen, preventing the interaction of the complex with the target cell.

Inhibition of lymphocyte cytotoxicity by hepatoma D23 antigen

A central role for tumour antigen in the inhibition of lymphocyte cytotoxicity is implied in the interpretation of the experiments on blocking of target cells by antigen–antibody complexes. Further experiments were therefore carried out to determine whether the cytotoxicity of lymphocytes from hepatoma D23-immune rats could be specifically inhibited by pre-exposure to solubilized hepatoma D23 antigen.

The tumour antigen preparations used in these analyses were again papain-solubilized hepatoma D23 cell membrane fractions (Baldwin and Glaves, 1972; Baldwin *et al.*, 1973*c*) characterized immunologically by assay of their capacity to neutralize syngeneic hepatoma D23 antibody in membrane immunofluorescence tests. Lymph node cells from hepatoma-immune, or in controls, normal rats, were incubated with increasing amounts of hepatoma D23 antigen for 45–60 minutes, then collected by centrifugation and resuspended in fresh tissue culture medium before addition to plated hepatoma cells in the microcytotoxicity test (Baldwin *et al.*, 1973*d*). As shown in Fig. 4, incubation of hepatoma D23 immune lymph node cells with hepatoma D23 antigen inhibited their cytotoxicity for cells of this tumour. This effect was clearly dependent upon the amounts of antigen added so that with 80 μg of the antigen fraction per 10^6 lymph node cells their cytotoxicity was completely inhibited,

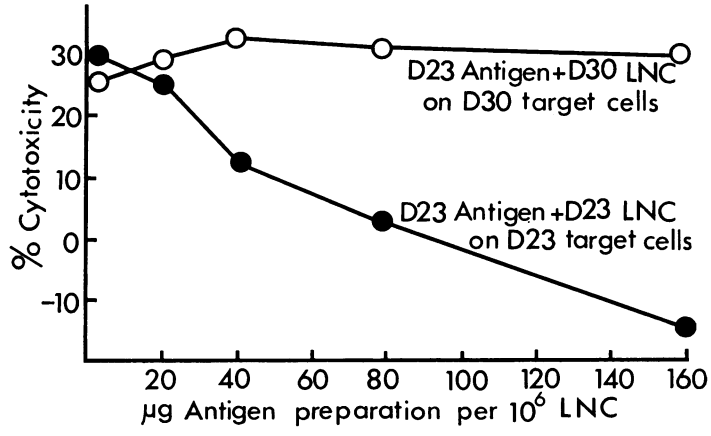

FIG. 4.—Inhibition of hepatoma D23 immune lymph node cell (LNC) cytotoxicity for hepatoma D23 target cells by solubilized hepatoma D23 membrane antigen. Pre-incubation of immune LNC with increasing amounts of solubilized hepatoma D23 antigen progressively reduced their cytotoxicity for hepatoma D23 cells. The specificity of the effect was controlled by comparable tests showing that hepatoma D23 antigen did not inhibit hepatoma D30 immune LNC for hepatoma D30 cells. In these tests, lymph node cells were incubated with hepatoma D23 antigen and this was removed before assay of their cytotoxicity for hepatoma D23 or D30 cells.

TABLE V.—*Cytotoxicity of Hepatoma Immune Lymph Node Cells (LNC) following Treatment with Serum from Tumour Bearer and Tumour Immune Rats*

Experiment	Target hepatoma	Serum donor	Percentage cytotoxicity	Percentage inhibition of cytotoxicity
1	D23	Normal	27·1	
		D23 immune	23·5	13·1
		D23 tumour bearer	4·4	83·7
2	D30	Normal	33·8	
		D30 immune	30·5	9·8
		D30 tumour bearer	11·9	64·8
3	D23	Normal	24·2	
		D23 immune	33·5	0
		D23 tumour bearer	−3·1	100
		D30 tumour bearer	25·2	0

whereas smaller amounts of the antigen preparation were progressively less effective. The tumour specificity of this effect was confirmed in control studies in which greater amounts of hepatoma D23 antigen preparation (160 $\mu g/10^6$ lymph node cells) did not inhibit the cytotoxicity of hepatoma D30 immune lymph node cells for plated hepatoma D30 cells.

Developing from these observations, experiments were performed to ascertain whether the cytotoxicity of lymphocytes from hepatoma immune rats could be specifically inhibited by pretreatment with tumour bearer serum. Representative data summarized in Table V indicate that pre-exposure of immune rat lymph node cells to serum from rats bearing the homologous tumour significantly inhibits their cytotoxicity for plated hepatoma target cells. Furthermore, this effect was specific to individual tumours since in cross tests, serum from rats bearing a hepatoma different to that used to produce sensitized lymphocytes was not inhibitory.

In comparable tests (Table V) pre-exposure of sensitized lymph node cells to serum from hepatoma immune rats did not influence their cytotoxicity. These antisera contain tumour specific antibody

detectable by membrane immunofluorescence staining of viable hepatoma cells (Baldwin and Barker, 1967*b*; Baldwin *et al.*, 1971) and by their complement dependent cytotoxicity for cultured hepatoma cells (Baldwin and Embleton, 1971). Moreover, these sera when complement inactivated can block plated hepatoma cells from lymph node cell cytotoxicity (Baldwin *et al.*, 1973*b*).

DISCUSSION

Abrogation of the *in vitro* cytotoxicity of sensitized lymphocytes for tumour cells may be effected in two ways: (a) cell surface expressed tumour antigens may be " masked " by interaction with antibody so preventing recognition by lymphocyte receptors; (b) lymphocytes may be " desensitized " by interaction with tumour associated antigen presented in an acellular form.

Considering first interactions at the level of the target cell *i.e.* " blocking " phenomena it is clear that tumour specific antibody can exert this effect as evidenced by the activity of sera from hepatoma immune rats (Baldwin *et al.*, 1973*b*). These antisera contain tumour specific antibody demonstrable by their complement dependent cytotoxicity (Baldwin and and Embleton, 1971) and by membrane immunofluorescence staining of cell surface antigens on viable hepatoma cells (Baldwin *et al.*, 1971). The blocking activity of hepatoma bearer serum is a more complex event, however, and free tumour specific antibody is not likely to be the primary effector, since these sera do not have significant levels of antibody as assayed by cytotoxicity assays (Table III), or by membrane immunofluorescence staining (Baldwin, Bowen and Price, 1973*a*). Data indicating that addition of hepatoma specific antibody to tumour bearer serum abrogates its blocking activity also argues against the involvement of antibody (Table IV).

The alternative possibility is that blocking activity is mediated by binding of tumour specific antigen–antibody complexes to tumour cells. In this case, the effect may again reflect steric blocking preventing lymphocyte attack, or alternatively the antigen moiety may have a specific role, interacting with the lymphocyte receptor and so inhibiting lymphocyte reactivity. It has already been established with rat hepatoma D23 that immune complexes prepared by the addition of tumour specific antibody to solubilized hepatoma D23 antigen can specifically block target cells from lymphocyte attack (Baldwin *et al.*, 1972). Conversely, Sjögren *et al.* (1971) established that the blocking factor in serum of Moloney sarcoma bearing mice can be dissociated at low pH and separated into high and low molecular weight fractions which individually lacked activity, this being restored on recombination. The implication from these studies was that the low and high molecular weight fractions were tumour antigen and antibody respectively and subsequent studies established that a similar complex could be eluted from human tumour cells (Sjögren *et al.*, 1972). More direct evidence for the presence of tumour specific immune complexes in the serum of rats bearing transplanted tumours has recently been reported (Thomson, Steele and Alexander, 1973; Baldwin *et al.*, 1973*a*). Using rat sarcomata, Thomson *et al.* (1973) demonstrated the presence of tumour specific antigen in the serum of tumour bearing rats by the capacity of insolubilized serum to absorb tumour specific antibody detected by membrane immunofluorescence. Antibody neutralization assays were also used to detect tumour specific antigen in immune complexes following fractionation of sarcoma bearer serum by membrane ultrafiltration at low pH or high salt concentration. Comparably, Baldwin *et al.* (1973*a*) isolated free tumour specific antigen from the serum of hepatoma bearing rats by Sephadex G-150 chromatography. The fraction excluded from the gel (molecular weight greater than 150,000) was then dissociated at pH 3·0 and fractionated by G-150 chromatography to yield hepatoma-

specific antibody demonstrable by its membrane immunofluorescence staining of viable hepatoma cells and hepatoma antigen, measured by its capacity to neutralize hepatoma specific antibody.

This view of blocking of lymphocyte cytotoxicity by tumour bearer serum being mediated by immune complexes is also implied in the " unblocking " phenomena where the addition of serum from tumour free individuals to tumour bearer serum neutralizes blocking activity (Hellström and Hellström, 1970*a*; Hellström *et al.*, 1971*a*). In this situation, however, immune complexes in the region of antibody excess should still be capable of attachment to the target cell antigen and provide at least as effective steric block as free antibody or immune complexes with free antigenic determinants. This implies that the blocking effect of tumour bearer serum may be dependent upon a specific desensitization of immune lymphocytes by the exposed antigen moiety of immune complexes bound at the tumour cell surface.

The evidence discussed so far has been considered from the point of view that antagonistic effects of humoral factors on cell mediated antitumour immunity are operative at the level of the target cell. If the crucial interaction during blocking at the target cell is binding of the antigen moiety to the sensitized lymphocyte, an alternative view is that desensitization of immune lymphocytes, either by immune complexes or free antigen, may be of paramount importance. As illustrated in Fig. 4, this effect can be directly demonstrated *in vitro* with hepatoma D23 by the specific reduction of lymphocyte cytotoxicity following addition of solubilized hepatoma D23 antigen (*cf.* also Baldwin *et al.*, 1973*d*). Comparably, serum from hepatoma D23 bearer rats also inhibits lymphocyte cytotoxicity following short term incubation of effector cells in serum (Table V). This view that circulating tumour specific antigen can directly inhibit lymphocyte cytotoxicity is compatible with the report of Brawn (1971) on the inhibition of alloantigen sensitized lymph node cells by normal mouse serum of the target cell strain, this being attributed to interaction with soluble serum borne histocompatibility antigens. Currie and Basham (1972) have also proposed that antigen desensitization may explain their finding that peripheral lymphocytes from melanoma patients with widely disseminated disease only became cytotoxic following repeated washing, presumably resulting in the elution of inhibitory factors. These inhibitory factors in tumour bearer serum have still to be characterized definitively but in the rat hepatoma system (Baldwin *et al.*, 1973*a*) there is conclusive evidence for the presence of circulating tumour specific antigen and immune complexes. That is, in animals with well established hepatomata the serum contains an excess of circulating tumour-specific antigen. A similar conclusion was reached by Thomson *et al.* (1973) who also showed that serum from rats with established sarcomata contains free circulating tumour specific antigen. Undoubtedly, however, proportions of free and immune complexed tumour antigen will vary at various stages of tumour growth. The levels of circulating immune complexes and free antigen, and also the strength of cellular immunity, may together form the basis of an evaluation of the immunological status of the host in relation to its tumour.

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