ADVANCES IN CANCER RESEARCH

VOLUME 20

ADVANCES IN CANCER RESEARCH

Edited by

GEORGE KLEIN

Department of Tumor Biology
Karolinska Institutet
Stockholm, Sweden

SIDNEY WEINHOUSE

Fels Research Institute
Temple University Medical School
Philadelphia, Pennsylvania

Consulting Editor

ALEXANDER HADDOW

Chester Beatty Research Institute
Institute of Cancer Research
Royal Cancer Hospital
London, England

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CONTRIBUTORS TO VOLUME 20

Numbers in parentheses refer to the pages on which the authors' contributions begin.

HEINZ BAUER,[1] *Robert Koch-Institut, Berlin, Germany* (275)

MAX M. BURGER, *Department of Biochemistry, Biozentrum, University of Basel, Basel, Switzerland* (1)

P. L. GROVER, *Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, England* (165)

SIR ALEXANDER HADDOW,[2] *Pollards Wood Research Station of the Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, England* (343)

MICHAEL H. LEVY, *Department of Microbiology, Thomas Jefferson University, Philadelphia, Pennsylvania* (131)

G. J. V. NOSSAL, *The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia* (93)

ANNETTE M. C. RAPIN, *Department of Biochemistry, Biozentrum, University of Basel, Basel, Switzerland* (1)

P. SIMS, *Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London, England* (165)

E. FREDERICK WHEELOCK, *Department of Microbiology, Thomas Jefferson University, Philadelphia, Pennsylvania* (131)

[1] Present address: Institut für Virologie, Bereich Human-Medizin, Frankfurter Strasse 107, 63 Giessen, West Germany.

[2] Present address: The Lodge, Pollards Wood Research Station, Nightingales Lane, Chalfont St Giles, Buckinghamshire HP8 4SN, England.

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Sir Alexander Haddow

TUMOR CELL SURFACES: GENERAL ALTERATIONS DETECTED BY AGGLUTININS

Annette M. C. Rapin and Max M. Burger

Department of Biochemistry, Biozentrum, University of Basel, Basel, Switzerland

I. Introduction

One of the most fundamental questions concerning malignant cells is: Why do they proliferate beyond normal limits and become invasive? This is the question posed in its most simple form by both layman and scientist.

Even though a host of researchers have been applying themselves to

this question for a number of years, it is still far from being answered. One concept that has emerged is that malignant cells show great alterations in their relationships, not only toward cells in the surrounding tissue which they may invade, but also toward each other. Kalckar (1965) has spoken of an altered "cell sociology" of malignant cells, stemming at least in part from their altered surface characteristics, and Pardee (1964) has pointed out that alteration of such membrane functions as transport could play an important part in control of growth.

Let us list briefly here some of the characteristics of tumor cells which are indicative of their having altered surface membranes:

A. *In the organism*

1. Tumor cells, whether they are part of a malignant or a benign tumor, multiply beyond normal limits, without being influenced by neighboring cells.
2. Most tumor cells do not cooperate with each other to form a tissue specialized for a certain function; individual cells thus become autonomous.
3. Although the chemical structure of cell surfaces is still poorly known, serological studies have shown the antigenic makeup of tumor cells to differ in several respects from that of normal cells:
   a. Appearance of tumor-specific transplantation antigens (TSTA) as well as virus-specific antigens on the surface of virally transformed cells (Klein, 1969),
   b. Appearance in many cases of carcinoembryonic antigens or oncofetal antigens (Abelev, 1971; Gold and Freedman, 1965a,b),
   c. Tumor cells are less differentiated than their normal counterparts; one manifestation of this is the decrease, or in some cases the total loss, of tissue-specific antigens (Weiler, 1959).

B. *In vitro.* The possibility of transforming cells *in vitro* (by oncogenic viruses, or by chemical carcinogens) or the ability to select spontaneously transformed cells allows a comparison between normal and transformed cells originally stemming from the same tissue.

1. A characteristic of untransformed tissue culture cells is their density-dependent inhibition of growth (DDI)[1] (Stoker and

[1] Abbreviations: cAMP, cyclic AMP = adenosine 3′,5′-monophosphate; CMP, cytidine 5′-monophosphate; cGMP, cyclic GMP = guanosine 3′,5′-monophosphate; cIMP, cyclic IMP = inosine 3′,5′-monophosphate; cUMP, cyclic UMP = uridine 3′,5′-monophosphate; DDI, density-dependent inhibition (usually of growth); diBcAMP, dibutyryl cAMP; DNP, dinitrophenol; Ig, immunoglobulin; PGE_1, prostaglandin E_1; RSV, Rous sarcoma virus; TAME, tosyl-arginine methyl ester (a protease inhibitor); TLCK, tosyl-lysyl-chloromethylketone (a protease inhibitor); TPCK, tosyl-phenylalanyl-chloromethylketone (a protease inhibitor). For abbreviations of lectin names, see Table I.

Rubin, 1967): At the stage where growing cells become confluent they cease dividing and do not continue to grow beyond the monolayer stage. Tumor cells escape this DDI of growth: contact does not prevent them from proliferating for a certain time, and they usually form disorganized multiple layers.

2. As first reported by Coman (1944) transformed cells are less adhesive toward each other and toward an external support. They can thus more easily grow in suspension, a property which in the organism would certainly be of advantage for their invasive and metastasizing growth (see also Abercombie and Ambrose, 1962).
3. Chemical and serological studies of surface membranes isolated from untransformed and from the corresponding transformed cells have shown a number of differences. Some of these studies will be briefly reviewed in Section VI.

C. *Increased agglutinability.* Based on some previous reports by Easty *et al.* (1960), Aub and his collaborators reported in 1963 that a lipase preparation from wheat germ agglutinated tumor cells preferentially to normal cells. Until that time phytohemagglutinins or lectins, proteins usually isolated from plant seeds, had been used mainly for blood typing and for carbohydrate studies. This rested on Boyd's demonstration in 1945 (see Boyd, 1970) that the specificity of various agglutinins for different blood groups depends on a specific reaction between the lectin and the blood group carbohydrates located on the surface of erythrocytes, which was reminiscent of antigen–antibody reactions. The differential behavior of normal and tumor cells with an agglutinin was another indication for the existence of surface differences between these cells, and this agglutinin reaction has become a very useful tool for the study of cell surfaces. Attempts to find out how the transition from a normal and nonagglutinable cell to one that is transformed and agglutinable takes place, should yield insights into molecular alterations and rearrangements occurring with the transformation process. Studies along these lines have been going on for the last few years in our own and in several other laboratories. A few questions have been answered, but many more have been raised.

This review will be concerned with studies on the agglutinin reaction, its use for the investigation of normal and of tumor cell surfaces, and with the process of transformation. It may not be amiss to stress at this point that this agglutination reaction is an artificial system since lectins are not present in the organism to interact with tumor cells. This should not detract from the usefulness of this reaction as a tool for the study of cell surfaces in general and of malignant transformation in particular.

It should be emphasized already at the beginning of this review that even though some correlations exist between changed surface characteristics of tumor cells and their neoplastic behavior, a causal relationship between these properties as well as an irrefutable proof of a direct involvement of the cell membrane in malignancy have yet to be conclusively demonstrated.

II. Correlation between Lectin Binding, Agglutinability, and the Transformed State

Following Aub's initial observation (Aub *et al.*, 1963) that a crude wheat germ lipase preparation selectively agglutinated malignant cells, the active principle from such preparations, the protein wheat germ agglutinin (WGA), was isolated (Burger and Goldberg, 1967). This lectin did indeed agglutinate leukemia cells and cells transformed by the tumorigenic polyoma virus better than the corresponding untransformed cells. From carbohydrate inhibition studies it was concluded that the reactive group at the cell surface, probably a glycoprotein, contained β-1,4-di-*N*-acetylglucosamine (Burger and Goldberg, 1967). These initial studies opened the way to a large number of investigations in many laboratories, using various plant agglutinins, such as concanavalin A (Inbar and Sachs, 1969), soybean agglutinin (Sela *et al.*, 1970), and a number of others (see Table I).

A review of these studies, which we shall present here, and which is summarized in Table I, leads to the conclusion that in general there is indeed a good correlation between tumorigenicity of cells and their ability to be agglutinated by plant lectins; like all generalizations, however, this one suffers a number of exceptions, and these will also be discussed here.

A. Agglutination Studies

Long before the discovery of the affinity of agglutinins for tumor cells, plant lectins were known to agglutinate red blood cells and Boyd found in 1945 (see Boyd, 1970) that they could be used for blood group typing [for historical review about this discovery, and for description of a number of lectins and their carbohydrate specificity, see Boyd (1970), Lis and Sharon (1973), and Sharon and Lis (1972)]. The ability of many lectins to agglutinate red blood cells from certain species shows that they are not exclusively tumor-specific (it should, however, be remarked in passing that human erythrocytes are not typical somatic cells). Aub himself followed his initial observations on the agglutinability of tumor cells (Aub *et al.*, 1963) by a series of tests on normal and tumor cells, and he found that although tumor cells consistently gave a

higher degree of agglutination than normal cells, the latter were often also agglutinated to a slight degree (Aub *et al.*, 1965a,b).

Although in the great majority of cases it is tumor cells and transformed cells that are agglutinated by lectins, a few cases have also been reported where the normal cells, not their transformed counterparts, agglutinated better. This, however, seems so far to be a particularity of the interaction of lentil (*Lens culinaris*) agglutinin with normal liver cells and with rat hepatoma cells (but not with other types of cells, Borek *et al.*, 1973; see also Section III,B,4).

In several instances no difference in agglutinability could be observed between normal and tumor cells (see Table I). In these cases transformation has not changed the availability of a certain receptor site; this, however, does not preclude modification of sites on the same cells for other lectins. An example of this phenomenon is the agglutinability of normal lymphocytes as well as lymphoma cells by the red kidney bean agglutinin PHA (*Phaseolus vulgaris* agglutinin), whereas concanavalin A (con A) agglutinates the lymphoma cells only (Inbar *et al.*, 1973a). It should also be kept in mind that different types of cells differ in their surface components and consequently in their ability to interact with different lectins [L1210 leukemia cells,[2] for instance, are very well agglutinated by WGA but not by Con A (Burger, 1973), whereas Yoshida sarcoma cells are agglutinated by Con A and by PHA, but poorly by WGA (Tomita *et al.*, 1972b); cells from a rat ascites hepatoma are agglutinable by WGA, but practically not by Con A (D. F. Smith *et al.*, 1973)].

Interesting are also the cases where cells have become agglutinable after infection with nononcogenic viruses: Infection of baby hamster kidney (BHK) cells by Newcastle disease virus resulted in a thinning of the cell coat and agglutinability of the cells by Con A and WGA; a viral mutant which did not produce this thinning of the cell coat did not render the infected cells agglutinable (Poste and Reeve, 1972). Becht *et al.* (1972) showed that cells infected with a variety of nononcogenic enveloped RNA viruses, such as Sindbis, vesicular stomatitis, or influenza virus, became agglutinable by Con A concomitantly with the appearance of hemagglutinin on their cell surface; cells infected in

[2] Description of cells mentioned in this review: 3T3, mouse embryo fibroblasts; Py3T3, polyoma virus-transformed 3T3 cells; SV3T3, simian virus 40 transformed 3T3; RSV3T3, Rous sarcoma virus-transformed 3T3; BHK (or BHK21), baby hamster kidney fibroblasts; CHO, Chinese hamster ovary cells (a dedifferentiated, epithelioid cell line); HeLa, a line derived from a human epithelioid carcinoma (cervical); KB, a line derived from a human carcinoma (oral), epithelioid; L cells, mouse fibroblasts; L1210, mouse leukemia cells; NIL, hamster fibroblasts.

TABLE I*

Some Agglutinins and Their Properties

(Numbers in parentheses correspond to references at bottom of table. Complete bibliographical references will be found at end of review.)

Part A

Lectin and source	Isolation and purification	Specific inhibitor	Agglutination			References	Other properties
			Normal cells (other than erythrocytes)	Normal cells + proteases or in mitosis	Transformed cells		
Concanavalin (Con A), from jack bean (*Canavalia ensiformis*)	Isolation, purification, and characterization (1, 17, 22, 38, 59, 60, 74, 75, 82)	α-D-Glucopyranosides, α-D-mannopyranosides, α-D-fructofuranosides (α-methylmannose generally used (29)	0, but binds to normal cells	+	+	5, 6, 8, 10, 12, 14–16, 19, 20, 25–28, 33, 36, 40, 42, 45, 48–50, 54, 61, 68, 71, 76–79, 83	Isolation of cell membrane receptor (3, 4, 72, 86)
	Crystallization (74, 75)		Some lines +, some lines −		Some lines +, some lines −	26, 45, 47, 66, 67	Agglutination is temperature dependent (35, 37, 39, 53, 80)
	MW of tetramer 100,000–120,000 (46, 82)				Temperature-sensitive mutants agglutinable only at permissive temperature	10, 13, 21, 53, 55, 65	Agglutination depends on low cellular ATP content (81)
	Con A not a glycoprotein (2, 59)				Cells having regained growth control are less agglutinable	16, 34, 41 62, 69	Con A is mitogenic (43, 44, 56, 64)
							Stimulation of lymphocytes by Con A raises cellular cGMP content (31)
							Lymphoma cells agglutinated better than normal lymphocytes (37)
							Membrane-bound Con A has relatively high mobility (70)

Normal, confluent cells + protein synthesis inhibitors are agglutinable	6, 12	Fetal cells are agglutinable (24, 48, 51, 73, 84)
		Cells infected with some nononcogenic RNA viruses are agglutinable (7, 11, 63, 76, 89)
Normal cells treated with urea are agglutinable	85	Covering of Con A sites on transformed cells (by monovalent Con A) restores growth control (14)
CHO cells converted to fibroblasts are less agglutinable	32	Agglutination depends on intact microtubules (9, 23, 88) Con A bound to lymphocytes restricts Ig receptor mobility (30, 87)
		Tumor cells incubated with Con A prior to injection are more tumorigenic and invasive (18)
		Con A does not block hamster egg fertilization (58)
		Binds to sea urchin egg and inhibits normal cell division (57)

* Parts A–F of Table I appear on pages 6–17.

(*Continued*)

Key to references for Table I,A:

1. Agrawal and Goldstein (1965)
2. Agrawal and Goldstein (1968)
3. Akedo *et al.* (1972)
4. Allan *et al.* (1972)
5. Arndt-Jovin and Berg (1971)
6. Baker and Humphreys (1972)
7. Becht *et al.* (1972)
8. Ben-Bassat *et al.* (1971)
9. Berlin and Ukena (1972)
10. Biquard and Vigier (1972a,b)
11. Birdwell and Strauss (1973)
12. Borek *et al.* (1973)
13. Burger and Martin (1972)
14. Burger and Noonan (1970)
15. Cline and Livingston (1971)
16. Culp and Black (1972)
17. Cunningham *et al.* (1972)
18. de Micco *et al.* (1973)
19. de Petris *et al.* (1973)
20. De Salle *et al.* (1972)
21. Eckhart *et al.* (1971)
22. Edelman *et al.* (1972)
23. Edelman *et al.* (1973)
24. Francois *et al.* (1972)
25. Friberg *et al.* (1971)
26. Friberg *et al.* (1972)
27. Furmanski *et al.* (1972)
28. Glick and Buck (1973)
29. Goldstein *et al.* (1965)
30. Gunther *et al.* (1973)
31. Hadden *et al.* (1972)
32. Hsie *et al.* (1971)
33. Inbar and Sachs (1969)
34. Inbar *et al.* (1969)
35. Inbar *et al.* (1971)
36. Inbar *et al.* (1972a)
37. Inbar *et al.* (1973a)
38. Jones and Johns (1916)
39. Kaneko *et al.* (1973)
40. Kapeller and Doljanski (1972)
41. Kurth and Bauer (1973)
42. Lehman and Sheppard (1972)
43. Lindahl-Kiessling (1972)
44. Mackler *et al.* (1972)
45. Mallucci (1971)
46. McKenzie *et al.* (1972)
47. Moore and Temin (1971)
48. Moscona (1971)
49. Nicolson (1971)
50. Nicolson (1972)
51. Noonan and Burger (1971)
52. Noonan and Burger (1973a)
53. Noonan and Burger (1973b)
54. Noonan *et al.* (1973a)
55. Noonan *et al.* (1973b)
56. Novogrodsky and Katchalski (1971)
57. O'Dell (1972)
58. Oikawa *et al.* (1973)
59. Olson and Liener (1967a)
60. Olson and Liener (1967b)
61. Ozanne and Sambrook (1971a)
62. Ozanne and Sambrook (1971b)
63. Poste and Reeve (1972)
64. Powell and Leon (1970)
65. Renger (1972)
66. Salzberg and Green (1972)
67. Salzberg and Raskas (1972)
68. Salzberg *et al.* (1973)
69. Schnebli and Burger (1972)
70. Shinitzky *et al.* (1973)
71. Shoham and Sachs (1972)
72. D. F Smith *et al.* (1973)
73. Steinberg and Gepner (1973)
74. Sumner (1919)
75. Sumner and Howell (1936)
76. Tevethia *et al.* (1972)
77. Turner and Burger (1974)
78. van der Noordaa *et al.* (1972)
79. Veselý *et al.* (1972)
80. Vlodavsky *et al.* (1972)
81. Vlodavsky *et al.* (1973)
82. Wang *et al.* (1971)
83. Weber (1973)
84. Weiser (1972)
85. Weston and Hendricks (1972)
86. Wray and Walborg (1971)
87. Yahara and Edelman (1972)
88. Yin *et al.* (1972)
89. Zarling and Tevethia (1971)

TABLE I (*Continued*)

Part B

Lectin and source	Isolation and purification	Specific inhibitor	Agglutination: Normal cells (other than erythrocytes)	Agglutination: Normal cells + proteases or in mitosis	Agglutination: Transformed cells	References	Other properties
Soybean agglutinin (SBA), from soybean (*Glycine max*)	Isolation, purification, and characterization (2, 5–9, 11, 17) MW of tetramer: 110,000 (6, 8, 11, 17) Is a glycoprotein (8, 17) Four isolectins isolated (7)	*N*-Acetyl-D-galactosamine (D-galactose is a weaker inhibitor) (10)	0, but binds to normal cells 0	+ +	+ Transformed hamster embryo cells agglutinated only after protease treatment	3, 12, 13 12	Agglutination is not temperature dependent (3, 4) Agglutination does not depend on cellular ATP content (16) Is not mitogenic (4) Normal lymphocytes and lymphoma cells are agglutinated to same degree (4) Trypsinization of erythrocytes increases binding and agglutinability (1, 15) Membrane-bound lectin has relatively low mobility (14)

Key to references for Table I,B:

1. Gordon *et al.* (1972a)
2. Gordon *et al.* (1972b)
3. Inbar *et al.* (1971)
4. Inbar *et al.* (1973a)
5. Liener and Pallansch (1952)
6. Lis and Sharon (1973)
7. Lis *et al.* (1966a)
8. Lis *et al.* (1966b)
9. Lis *et al.* (1969)
10. Lis *et al.* (1970)
11. Pallansch and Liener (1953)
12. Sela *et al.* (1970)
13. Sela *et al.* (1971)
14. Shinitzky *et al.* (1973)
15. Vlodavsky *et al.* (1972)
16. Vlodavsky *et al.* (1973)
17. Wada *et al.* (1958)

TABLE I (*Continued*)

Part C

Lectin and source	Isolation and purification	Specific inhibitor	Agglutination			References	Other properties
			Normal cells (other than erythrocytes)	Normal cells + proteases or in mitosis	Transformed cells		
Wheat germ agglutinin (WGA), from wheat germ (*Triticum vulgaris*)	Isolation, purification, and characterization (1, 2, 8, 10, 25, 27, 30–32, 40)	Di-*N*-acetylchitobiose; *N*-acetylglucosamine; ovomucoid (2, 8, 10, 16, 45)	0, but binds to normal cells	+	+	3–5, 8–10, 13, 14, 17, 19, 22–24, 29, 35, 36, 39 41, 46, 48	Isolation of cell membrane receptor (7, 21, 44, 50)
	Crystallization (30, 32)		Some lines +, some lines −		Some lines +, some lines −	4, 15, 26, 28	Agglutination not temperature dependent (19, 20)
	MW of dimer: 35,000 (2, 31)				Temperature-sensitive mutants agglutinable only at permissive temperature	11, 12, 33, 38	Agglutination not dependent on cellular ATP content (47)
	Not a glycoprotein (1, 2, 31)						Nonmitogenic (20)
	Three isolectins separated (2, 27)				Cells having regained growth control are less agglutinable	36, 39	Normal lymphocytes and lymphoma cells agglutinated to same degree (20)
			Normal, confluent cells + protein synthesis inhibitor are agglutinable			6	Membrane-bound lectin has relatively low mobility (43)