Recognition sites involved in the adhesive processes of neoplastic and normal homogeneic cells[1]

P. Pippia[2], A. Cogoli, G. Tilloca and G. Ivaldi

Istituto di Fisiologia Generale dell'Università di Sassari, Via Muroni 25, I-07100 Sassari (Italy), and Laboratorium für Biochemie, ETH-Zentrum, CH-8006 Zürich (Switzerland), October 11, 1982

Summary. Cross-adhesion experiments between neoplastic and normal homogeneic cells show that trypsin-sensitive recognition sites are involved in cell-cell adhesion of neoplastic cells and that the affinity between heterologous recognition sites is higher then between homologous ones.

A critical comparison of several studies of cellular adhesion performed in the last years must take into account the experimental methodology employed. In fact there are substantial differences between the molecular mechanisms which regulate cell-substrate adhesion and those involved in intercellular adhesion[3,4]. For instance, Di Pasquale observed that in cell-substrate adhesion the adhesive bridges are arranged over the whole cell surface[5] while in cell-cell adhesion the bridges are localized at the periphery of the cell only[6]. It is known that neoplastic cells or cells transformed by oncogenic viruses have no anchorage dependence at all, or a very much reduced one. This depends on the fact that the transformation either reduces the number of the cell-substrate contacts or makes then less efficient[7,8]. According to these features, the contact-inhibition of movements of normal cells is explained as a consequence of the formation of stable cell-substrate contacts which reduce cell motility[9]. Conversely, in transformed cells where cell-substrate contacts are more labile, contact-inhibition of movements is weakened, i.e. cells are free to migrate and to move over each other[6]. Recently, while investigating a number of functional groups of the coat of neoplastic (SGS-2) and normal (FG) cells involved in cell-cell adhesion[10,11], we observed that neoplastic cells have a remarkably higher adhesive capacity than homogeneic fibroblasts and that the adhesion kinetics may differ considerably depending on the disaggregating agent (either trypsin or EDTA) used for preparing the single cells. Based on these observations, we decided to perform a study with the objective of establishing whether the higher adhesiveness of SGS-2 cells is a property related to certain components of their cell coat or to other cell properties.

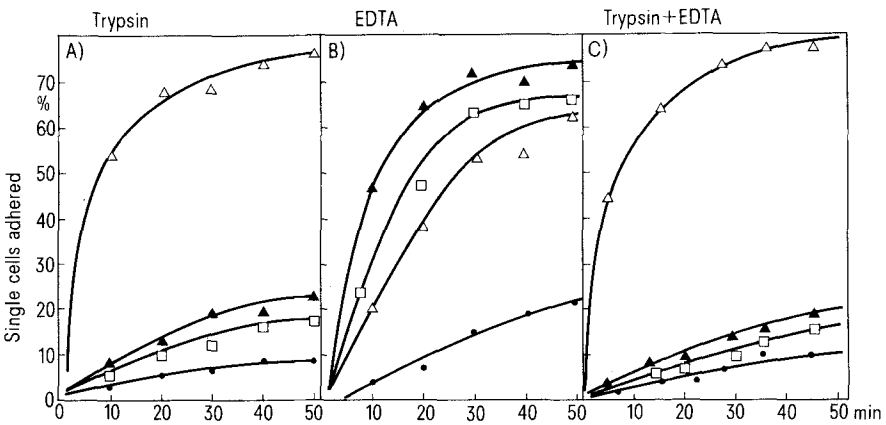
Materials and methods. The neoplastic cells used in this study (SGS-3A) were derived from the SGS-2 (Sarcoma Galliera Strain)[12] by 3 consecutive clonings on semisolid substrate according to the method of Macpherson[13], in order to eliminate fibroblasts usually present in the culture. The normal fibroblast strain (FG) was derived from embryos of the same rat strain. Cells were grown in Dulbecco's modified MEM supplemented with 10% newborn calf serum. A modification of the adhesion assay of Roseman et al.[10,14] was used to measure the attachment of L-4,5-^{3}H leucine-labeled single cells (Amersham, 61 Ci/mmole) to confluent monolayers cultured in multi-well Linbro plates (Flow Laboratories). Cell viability was determined by trypan blue dye exclusion. Student's 2-tailed t-test was used as test of significance in comparing ARC values.

Results and discussion. Results of experiments to determine the extent of homologous and heterologous adhesion between FG normal (N) and SGS-3A tumor (T) cells are shown as adhesion kinetics in the figure and summarized as mean ARC (adhesion rate constant) values in the table. Experiments with trypsin-released cells (fig. A) demonstrate that the homologous adhesion has rather low ARC values, while the heterologous adhesion kinetic T/N has an ARC value which is 1.25 times ($p < 0.05$) higher than that of the homologous adhesion T/T. In contrast, the ARC value of the adhesive system N/T is remarkably higher than that of the 2 homologous adhesive systems, 4.5 times ($p < 0.001$)

Comparison of adhesion rate constants in homologous and heterologous intercellular adhesion between normal (N) and tumor (T) cells

Adhesion type	Dissociating agent		
	Trypsin	EDTA	Trypsin + EDTA
N/N	0.2 ± 0.03	0.4 ± 0.03	0.2 ± 0.04
T/T	0.4 ± 0.05	1.6 ± 0.09	0.3 ± 0.05
T/N	0.5 ± 0.05	1.8 ± 0.12	0.4 ± 0.05
N/T	1.85 ± 0.15	1.4 ± 0.1	2.0 ± 0.21

The values represent the mean adhesion rate constants (± SD) from 4 experiments. The ARC value was measured in the linear part of the cell-cell adhesive curve and is defined as the percentage of single cells in the suspension that adhered to the monolayer[15].



Time-course of homologous N/N (●–●) and T/T (□–□) and heterologous N/T (△–△) and T/N (▲–▲) intercellular adhesion. In each experiment 0.33 ml of single labeled cells suspension (8×10^4–1.2×10^5 cells/ml) were added to 1 cm^2 of cellular monolayer and incubated at 37 °C. Single labeled cells were prepared with 0.25% trypsin (A) or with 0.5 mM EDTA (B) or with a mixture of 0.02% EDTA and 0.05% trypsin (C). The adhesion is expressed as percent of single labeled cells adhering to a homologous or heterologous monolayer.

higher than T/T and 9 times ($p < 0.001$) higher than N/N, approximately.

In experiments with EDTA-released cells (fig. B) the heterologous adhesive system N/T again retains a high ARC value, as observed in the same system with trypsin-released cells. Conversely, in the systems T/T and T/N the ARC values are even higher, 1.14 ($p < 0.05$) and 1.28 times ($p < 0.01$) respectively, than that of the N/T system; finally, in the adhesive system T/N the ARC value is 1.13 times ($p < 0.05$) higher than that of T/T system. Adhesion kinetics of cells released with a mixture of EDTA and trypsin (fig. C) are almost identical to those observed with trypsin-released cells. This result indicates that the differences found in the 2 categories of experiments can be attributed to the specific action of trypsin rather than to that of EDTA.

From the analysis of our results we conclude the following; a) the adhesion sites of neoplastic cells are more sensitive to trypsin than those of fibroblasts; b) there is a higher affinity of adhesion between tumor and normal cells, and between tumor cells themselves, compared to the homologous adhesion between normal cells (this could depend on some structural properties of the adhesion sites). Moreover, the data reported suggest the possibility that the density of adhesive sites on neoplastic cells may be higher than that on fibroblasts.

The interpretation of our results agrees with the observation of Hahn and Yamada[16] suggesting that the effect of trypsin on fibronectin, the glycoprotein responsible for the adhesion, consists of the lysis of a particularly sensitive portion. A higher sensitivity to trypsin in neoplastic cells could either be due to a biosynthetic defect or to the particular assembly of the constituents of the adhesion sites. An altered biosynthesis could involve the carbohydrate moiety, i.e. that portion of a glycoprotein which makes it resistant to proteases[17].

1 This research was supported in part by the Italian CNR Rome, grant No. 79.00955.04, and in part by the Swiss National Science Foundation, Berne, grant No. 3.499.079. The skillful technical assistance of Mrs Mirjam Morf Valluchi is gratefully acknowledged.

2 Reprint request to: P. Pippia, Istituto di Fisiologia Generale, Via Muroni 25, I-07100 Sassari (Italy).

3 Weinstein, R.S., Merck, F.B., and Alroy, J., Adv. Cancer Res. *23* (1973) 23.
4 Culp, L.A., Curr. Topics Membrane Transp. *11* (1978) 327.
5 Di Pasquale, A., Exp. Cell Res. *94* (1975) 191.
6 Di Pasquale, A., and Bell, P.P., J. Cell Biol. *62* (1974) 198.
7 Culp, L.A., and Black, P.H., Biochemistry *11* (1972) 2161.
8 Shields, R., and Pollock, K., Cell *3* (1974) 31.
9 Martz, E., Philips, H.M., and Steinberg, M.S., J. Cell Sci. *16* (1974) 401.
10 Pippia, P., Ivaldi, G., and Cogoli, A., FEBS Letters *116* (1980) 281.
11 Pippia, P., Cogoli, A., Gaias, M., Piras, G., and Ivaldi, G., Experientia *37* (1981) 698.
12 Pippia, P., Tilloca, G., Vargiu, F., Cherchi, G.M., Coinu, R., and Ivaldi, G., Pathologica *70* (1978) 19.
13 Macpherson, I., and Montagnier, L., Virology *23* (1964) 291.
14 Roseman, S., Rottman, W., Walther, B., Öhman, R., and Umbreith, J., Meth. Enzymol. *32* (1974) 597.
15 Walther, B., Öhman, R., and Roseman, S., Proc. natl Acad. Sci. USA *70* (1973) 1569.
16 Hahn, L.H.E., and Yamada, K.M., Cell *18* (1979) 1043.
17 Olden, K., Pratt, R.M., and Yamada, K.M., Cell *13* (1978) 461.

0014-4754/83/121396-02$1.50 + 0.20/0

Another new kind of *Chlamydomonas* mutant, with impaired flagellar autotomy[1]

R.A. Lewin and C. Burrascano[2]

Marine Biology Research Division, Scripps Institution of Oceanography, La Jolla (California 92093, USA), March 4, 1983

Summary. We have obtained a UV-induced mutant (designated *fa*−) of *Chlamydomonas reinhardtii*, in which the mechanism of flagellar autotomy is impaired. Whereas wild-type cells normally shed their flagella in 17% ethanol, for instance, the mutant cells retain them.

Flagellate cells of algae shed their flagella when stressed. The reaction seems to be general among chlorophytes, dinoflagellates, euglenophytes, and probably most algae of other classes. It is induced by sublethal extremes of temperature or pH, by detergents, alcohols and other irritants. Detachment of the flagella is rapid, being often completed in less than 1 sec (at 20–25 °C). It occurs at a specific site distal to the basal body, just above the transition region. If the cell survives the shock, it can grow new flagella in an hour or so. The process of re-growth has been much studied, chiefly in *Chlamydomonas*, but the shedding process has received relatively little attention. It is an active process; it is not simply a breaking-off due to mechanical forces acting on a fragile organelle. Flagella are ordinarily tough and firmly attached, and cannot easily be detached from dead ('fixed') cells.

If autotomy of flagella is an active biological process, as the evidence indicates, it presumably plays some role in cell or species survival. This point was briefly discussed by Lewin et al.[3]. One may postulate that it must therefore be under genetic control, and it should therefore be possible to impair the mechanism by genetic damage. In fact, by mutagenesis we have obtained a non-autotomic mutant of *Chlamydomonas* in which the cells do not shed their flagella when stressed. We postulate the effect is attributable to a mutated gene which we are designating *fa*− (indicating impaired flagellar autotomy). Mutant cells retain sexuality, and have been crossed with *fa*+ (normally autotomous)