Vol. 88, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 13, 1979 Pages 818-825

DECREASED SENSITIVITY OF AN INTERFERON-RESISTANT SUBLINE OF MURINE LEUKEMIA L-1210 CELLS TO TOXIC EFFECTS OF RICIN AND ABRIN

Françoise Besançon* and Helmut Ankel

Department of Biochemistry, Medical College of Wisconsin
Milwaukee, Wisconsin 53226

Received April 10,1979

Summary: Approximately two to four times higher concentrations of the plant toxins abrin and ricin are required to inhibit protein synthesis in interferon-resistant L-1210R cells to the same degree as in interferon-sensitive L-1210S cells. However, amounts of interferon 10-fold higher than those required for protection from viral infection fail to show any effect on ricin intoxication of L-1210S cells.

Recent evidence suggests that interferon interacts with carbohydrate-containing cell membrane constituents. Its antiviral action is blocked by plant lectins (1) and after preincubation with gangliosides (2-4). Gangliosides covalently attached to Sepharose avidly bind both mouse and human interferons (3,4), which can be eluted with N-acetyl-neuraminyl lactose, the trisaccharide common to many gangliosides (3,5). Preincubation of SV/ALN cells with gangliosides under conditions that lead to incorporation into the cell membrane of these cells, increases their sensitivity to interferon (4). These observations suggest that gangliosides or related membrane constituents are part of the interferon receptor on the cell membrane.

Binding of the plant toxins abrin and ricin to carbohydrate-containing structures on the cell membrane, which appear to be glycoprotein constituents, precedes their toxic action (6). However, it has been shown repeatedly that ricin, like interferon, can also bind to gangliosides (7-9).

By culturing mouse leukemia L-1210 cells in the presence of interferon, Gresser, Bandu and Brouty-Boye have isolated a sub-population resistant to its anticellular and antiviral actions (10). Whereas significant amounts of inter-

*Visiting Scientist from Institut National de la Santé et de la Recherche Médicale, Unité 43, Hôpital Saint Vincent de Paul, Paris. Present address: Laboratoire de Biochimie Physique, I.N.R.A., Université Paris-Sud, 91 405 Orsay, France

0006-291X/79/110818-08$01.00/0

feron could be recovered from extracts of interferon-treated interferon-sensitive L-1210 cells, extracts of equally treated interferon-resistant L-1210 cells were devoid of antiviral activity (10). These results suggested that failure of resistant L-1210 cells to respond to interferon might be caused by a lack of binding and (or) uptake of interferon molecules.

If interferon shares common cell membrane receptors with abrin and ricin which are altered in the L-1210 subline devoid of interferon sensitivity, one might expect that the toxic effects of both toxins on these cells would also be decreased. In this communication we demonstrate that this is indeed the case. However we were unable to show protection of L-1210S cells from ricin intoxication by comparable concentrations of interferon.

MATERIALS AND METHODS

Cells: Interferon-sensitive and resistant L-1210 cells (L-1210S and L-1210R cells) were kindly provided by Dr. Ion Gresser. They were grown in RPMI 2310 medium supplemented with 5% fetal bovine serum (Gibco).

Reagents: Mouse interferon was purchased from Bionetics or was a gift from Dr. Peter Lengyel. The specific activities were 10^4 and 10^7 NIH Reference Units per mg protein (IU/mg), respectively. Toxins from Ricinus communis (ricin 60) and from Abrus precatorius (abrin) were purchased from P-L Biochemicals and from Sigma, respectively.

Interferon assay: L-1210S or L-1210R cells (2×10^6) were incubated at 37° for 5 or 18 hours with 10^3 IU of interferon in 1 ml RPMI medium. In parallel control cells were incubated with medium only. The cells were then infected with Vesicular Stomatitis Virus (VSV) at a multiplicity of infection of 0.1 plaque-forming units (PFU) per cell. After 18 hrs at 37°, the viral yield was determined by plaque assay.

Toxicity of ricin and abrin: This was determined by measuring inhibition of protein synthesis in the presence of the toxins (11). L-1210S or L-1210R cells (2×10^6) were suspended in 1 ml of RPMI medium without serum containing different concentrations of the toxins. Control cells were suspended in RPMI medium without toxin. After 3 or 18 hours at 37°, 0.5 µCi of [^{14}C]leucine was added to each sample. After further incubation for one hour, the radioactivity incorporated into cell proteins was measured according to the following procedure: the cells were washed twice with one ml of phosphate-buffered saline (PBS), then one ml of 0.3% sodium dodecyl sulfate was added to each sample. After 30 minutes at 37°, proteins were precipitated by adding one ml of 20% trichloracetic acid (TCA) and the samples were held for one hour at 4°. The TCA-insoluble material was then collected on Whatman GF/C filters which were washed with 5% TCA and methanol. The radioactivity retained on the filters was measured in a scintillation counter.

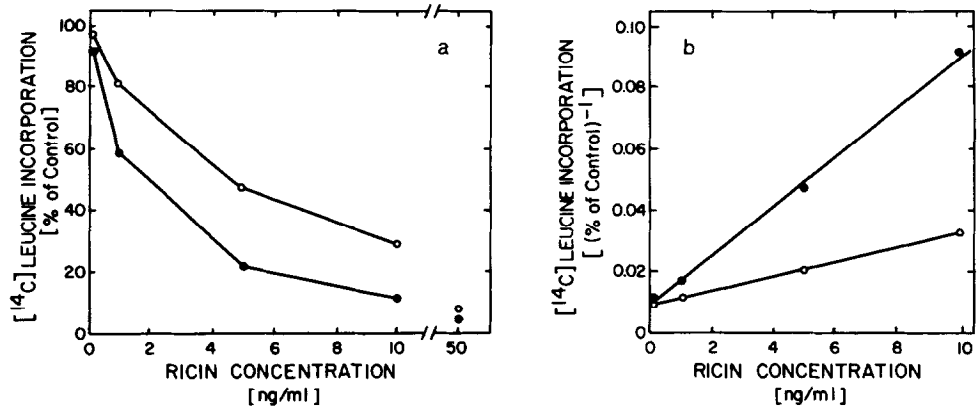

Figure 1A (left): Inhibition of [^{14}C]leucine incorporation into protein after 3 hours of preincubation with the indicated concentrations of ricin. Open circles: L-1210R cells; closed circles: L-1210S cells. The assays were carried out as described in Materials and Methods.

Figure 1B (right): Plot of the reciprocal of [^{14}C]leucine incorporation against ricin concentration. Data are the same as in Figure 1A. Open circles: L-1210R cells; closed circles: L-1210S cells.

RESULTS AND DISCUSSION

When L-1210S or L-1210R cells were preincubated for 3 hours with increasing amounts of ricin, protein synthesis in both cell lines was progressively inhibited (Figure 1A). However, to obtain the same degree of inhibition as in L-1210S cells, L-1210R cells required considerably higher concentrations of toxin. A reciprocal plot of the relative amounts of [^{14}C]leucine incorporated into protein versus ricin concentration results in straight lines (Figure 1B). Values for 50% inhibition of protein synthesis, derived from these plots, are 1.2 ng/ml for the L-1210S cells and 4.6 ng/ml for the L-1210R cells. Preincubation of both cell lines with ricin for 18 hours required much lower concentrations to obtain comparable inhibition of protein synthesis (Figure 2). Furthermore, the difference in toxin sensitivity of both cell lines was even more pronounced. Thus, whereas 0.01 ng/ml ricin inhibited protein synthesis of L-1210S cells by 25%, the same concentration was practically without effect on L-1210R cells.

Comparable results were obtained with the related toxin abrin. However, much lower concentrations of abrin were sufficient to produce measurable

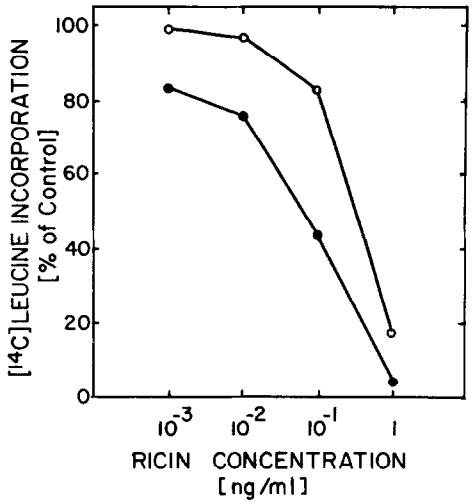

Figure 2: Inhibition of [^{14}C]leucine incorporation into protein after 18 hours of preincubation with the indicated concentrations of ricin. Open circles: L-1210R cells; closed circles: L-1210S cells. The assays were carried out as described in Materials and Methods.

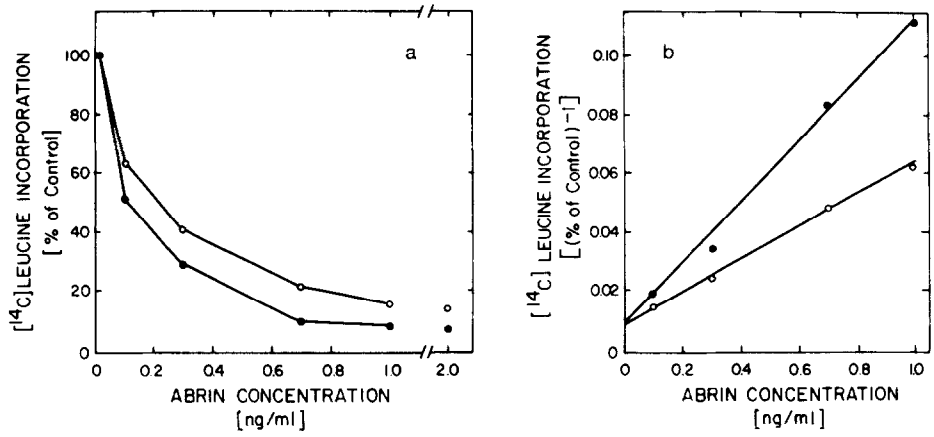

Figure 3A (left): Inhibition of [^{14}C]leucine incorporation into protein after 3 hours of preincubation with the indicated concentrations of abrin. Open circles: L-1210R cells; closed circles: L-1210S cells. The assays were carried out as described in Materials and Methods.

Figure 3B (right): Plot of the reciprocal of [^{14}C]leucine incorporation against abrin concentration. Data are the same as in Figure 3A. Open circles: L-1210R cells; closed circles: L-1210S cells.

inhibition of protein synthesis in both cell lines. Furthermore, the difference in sensitivity towards this toxin between L-1210S and L-1210R cells was somewhat less striking than in the case of ricin, both after 3 hours (Figure 3A) and after 18 hours (Figure 4). Straight lines obtained by plot-

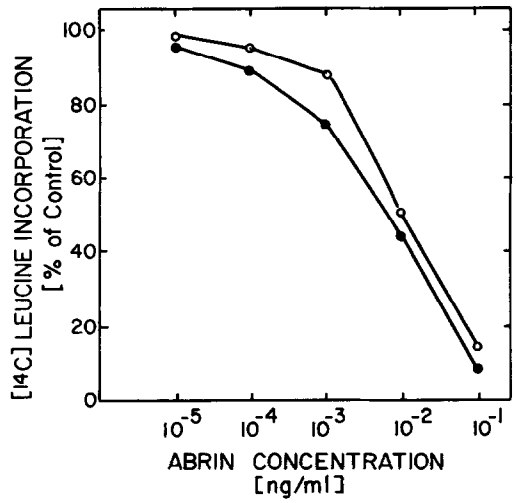

Figure 4: Inhibition of [^{14}C]leucine incorporation into protein after 18 hours of preincubation with the indicated concentrations of abrin. Open circles: L-1210R cells; closed circles: L-1210S cells. The assays were carried out as described in Materials and Methods.

ting the reciprocal of the relative amounts of [^{14}C]leucine incorporated into protein versus abrin concentration for the 3 hour period extrapolate to 0.1 ng/ml abrin for 50% inhibition of protein synthesis in the L-1210S cells, and to 0.2 ng/ml in the L-1210R cells (Figure 3B).

In order to investigate whether interferon competes with ricin for common receptors, L-1210S cells were incubated with mouse interferon prior to the addition of ricin. As seen in Table 1, no effect of interferon on inhibition of protein synthesis by ricin was observed, suggesting that at least under the conditions of the experiment interferon was not competitive with ricin. Assuming a molecular weight of 35,000 and a specific activity of 2.4 x 10^9 IU/mg for homogeneous mouse interferon (12), the 10,000 IU/ml employed in the experiment correspond to a concentration of 1.2 x 10^{-10} M, which is more than twice the concentration of ricin that was used (molecular weight 65,000, ref. 6). Thus if both substances had comparable affinities for common receptors, interferon should have blocked toxin action, at least partially. One tenth of the amount of interferon employed almost completely inhibited VSV

TABLE 1

Inhibition of [^{14}C]leucine incorporation by ricin in the absence and in the presence of interferon

Experiment	Addition to culture medium	[^{14}C]leucine incorporated cpm	% of control
I	Medium	4750	100
II	Interferon	4650	98
III	Ricin	1450	31
IV	Interferon, then ricin	1410	30

L-1210S cells were preincubated with 10,000 IU/ml interferon in II and IV and with medium in I and III for 80 minutes at 37°. Then 10 µl of a solution containing 3 ng of ricin were added to III and IV and incubation was continued for another 3 hours. [^{14}C]leucine incorporation was then determined as described in Materials and Methods.

TABLE 2

Viral yield after incubation of L-1210S and L-1210R cells with interferon

Cells	Pretreatment	Viral Yield [PFU/ml x 10^{-5}]
L-1210S	5 hrs medium	14
	5 hrs interferon	0.19
	18 hrs medium	50
	18 hrs interferon	0.07
L-1210R	5 hrs medium	15
	5 hrs interferon	25
	18 hrs medium	55
	18 hrs interferon	60

Antiviral assays were carried out as described in Materials and Methods.

multiplication in L-1210S cells, and as expected from the original observations by Gresser et al. (10), was without antiviral effect on L-1210R cells (Table 2).

A variety of toxins including those investigated here, and diphtheria, cholera and tetanus toxins are composed of two types of subunits: one responsible for binding to specific cell membrane receptors, the other for the biological effect of each individual toxin (6,13-15). Receptors for these toxins

appear to be glycoproteins (abrin, ricin, diphtheria toxin; ref. 6,16), or glycolipids (cholera and tetanus toxins; ref. 14,15). However, at least ricin has been found to bind to gangliosides as well (7-9). Because interferon has previously been shown to interact with gangliosides, our observation that interferon-resistant L-1210 cells are also less sensitive to the toxic effects of ricin and abrin suggests that this phenomenon might be due to changes of gangliosides or ganglioside-like cell-membrane constituents involved in productive binding of both interferon and plant toxins. However, since under our experimental conditions interferon did not protect L-1210S cells from intoxication by ricin, the reason for partial resistance of L-1210R cells towards plant toxins could also involve other alterations. Since the plant toxin A-chains act by inactivating ribosomes (17), one possibility is that growth in the presence of interferon also selects for ribosomal mutants that are less sensitive to toxin-induced inhibition of protein synthesis.

Preliminary experiments with [^{14}C]glucosamine-labeled cells indicate that chloroform/methanol-extractable gangliosides from both L-1210R and L-1210S cells have identical mobilities in TLC and are present in comparable ratios (unpublished observation). However, since we have not carried out detailed structural analyses of these gangliosides, the possibility of structural differences not manifested in changes in chromatographic mobilities cannot be ruled out. Such studies are in progress.

ACKNOWLEDGEMENTS

This work was supported by NSF grant PCM75-03117. We thank Dr. Ion Gresser for sending us L-1210S and L-1210R cells and Dr. Peter Lengyel for supplying us with partially purified mouse interferon.

REFERENCES

1. Besançon, F., and Ankel, H. (1974) Nature (London) 250, 784-786.
2. Besançon, F., and Ankel, H. (1974) Nature (London) 252, 478-480.
3. Besançon, F., Ankel, H., and Basu, S. (1976) Nature (London) 259, 576-578.
4. Vengris, V.E., Reynolds, F.H., Hollenberg, M.D., and Pitha, P.M. (1976) Virology 72, 486-493.
5. Besançon, F., and Ankel, H. (1977) Texas Rep. on Biol. and Med. 35, 282-292.
6. Olsnes, S., and Pihl, A. (1977) in Receptors and Recognition, Series B, Vol. 1 (P. Cuatrecasas, ed.) pp 129-173, Halsted Press, New York.

7. Surolia, A., Bachhavat, B.K., and Podder, S.K. (1975) Nature (London) 257, 802-804.
8. Boldt, D.H., Speckart, S.F., Richards, R.L., and Alving, C.R. (1977) Biochem. Biophys. Res. Comm. 74, 208-214.
9. Surolia, A., and Bachhavat, B.K. (1978) Biochem. Biophys. Res. Comm. 83, 779-785.
10. Gresser, I., Bandu, M.T., and Brouty-Boyé, D. (1974) J. Natl. Cancer Inst. 52, 553-559.
11. Sandvic, K., Olsnes, S., and Pihl, A. (1976) J. Biol. Chem. 251, 3977-3984.
12. De Maeyer-Guignard, J., Tovey, M.G., Gresser, I., and De Maeyer, E. (1978) Nature (London) 271, 622-625.
13. Collier, R.J. (1977) in Receptors and Recognition, Series B, Vol. 1 (P. Cuatrecasas, ed.) pp 69-98, Halsted Press, New York.
14. Bennett, V., and Cuatrecasas, P. (1977) ibid. pp 3-66.
15. Bizzini, B. (1977) ibid. pp 175-218.
16. Proia, R.L., Hart, D.A., Holmes, R.K., Holmes, K.V., and Eidels, L.R. (1979) Proc. Natl. Acad. Sci. (USA) 76, 685-689.
17. Olsnes, S., Sandvic, K., Refsnes, K., and Pihl, A. (1976) J. Biol. Chem. 257, 3985-3992.