Antibodies Produced by Immunization of Goats with 40S Ribosomal Subunits from Novikoff Hepatoma Ascites Cells[1] (36605)

ROSE K. BUSCH, WILLIAM H. SPOHN, YERACH DASKAL, AND HARRIS BUSCH

Tumor By-Products Laboratory, Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77025

In a previous report from this laboratory (1), it was found that goats immunized with 60S ribosomal subunits isolated from Novikoff hepatoma ascites cells (NHAC) developed agglutinins for these cells that also suppressed tumor growth. Moreover, antisera to liver 60S ribosomal subunits did not contain any agglutinins for the Novikoff tumor cells. In earlier studies Lamon and Bennett (2) produced precipitins in rabbits to mouse tumor ribosomes and Noll and Bielka (3, 4) produced antibodies to rat and bovine liver ribosomes in rabbits. However, no report of antitumor activity was presented in their studies. Since *in vitro* agglutination with antisera for 60S ribosomal subunits resulted in suppression of tumor growth *in vivo*, the present study was designed to determine whether goat antisera to 40S ribosomal subunits exhibited similar properties of agglutination of tumor cells and suppression of tumor growth.

Materials and Methods. The agglutination studies were carried out with antisera from goats immunized with the 40S ribosomal subunits from the Novikoff hepatoma ascites cells. The methods for isolation of the subunits and for preparation of the antisera were the same as those described previously (1): Pelleted polysomes were suspended in 0.005 *M* tris–HCl, pH 7.4, with gentle homogenization to give a concentration with an absorbance at 260 nm of 30 OD/ml. The suspension was made 0.008 *M* with respect to EDTA, layered on a 5–45% sucrose gradient buffered with 0.01 *M* tris–HCl, pH 7.4, and centrifuged for 16 hr in a Beckman BIV rotor at 70,000*g*. The 40S and 60S fractions were collected and pelleted in a Beckman 60 Ti rotor for 8 hr at 240,000*g*. The cells used were Novikoff hepatoma ascites cells, Walker 256 carcinosarcoma ascites cells and white cells from the rat spleen. To remove most of the red blood cells, the cells were washed three times in 0.15 *M* NaCl centrifuged at 400*g* for 3–4 min and resuspended in saline so that 1 ml of cell suspension contained approximately 10^7 cells. The cell suspensions and antisera were incubated in a 37° water bath for 1 hr and the tubes were shaken every 10–15 min. A drop of cell suspension was examined microscopically after the incubation period. The percent agglutination was determined from the proportion of cells in clusters to the total counted. Details of these methods were reported previously (1).

Immunofluorescence. The Novikoff hepatoma ascites tumor cells were incubated with antiserum to the 40S ribosomal subunits for 1 hr in a 37° water bath. Following incubation, the cells were washed three times with 0.15 *M* NaCl and reincubated with 0.1 ml fluorescein-labeled rabbit antigoat γ-globulin. The cells were washed three times to remove the labeled antiserum, resuspended in phosphate buffered glycerol (pH 7.2) and examined with a fluorescence microscope.

Studies on tumor growth. In these experiments, Novikoff hepatoma ascites tumor cells were obtained from 25 ml of peritoneal fluid for each group of ten rats. The cells were pelleted, washed in NKM solution (0.13 *M* NaCl, 0.005 *M* KCl and 0.008 *M* $MgCl_2$), pelleted again and 5×10^8 cells were resuspended either in 25 ml of antiserum to the 60S ribosomal subunits, 25 ml of antiserum to the 40S ribosomal subunits, or a combina-

[1] Supported by U.S. Public Health Service Grant CA-10893, the Welch Foundation grant Q-272 and the American Cancer Society Grant NP-28F.

tion of both, *i.e.*, 12.5 ml of each antiserum. In some experiments, the amount of antisera to the 40S and 60S ribosomal subunits was increased to 50 ml and in others, 25 ml each of antisera to the 60S and 40S ribosomal subunits were combined. Normal serum in equivalent amounts served as a control. The tumor cells were incubated with the respective antisera for 2 hr at 37° and the tubes were shaken every 20 min.

Following the incubation, the cells and serum were centrifuged at 400*g* for 3–4 min and the supernatant serum was discarded. The pelleted cells of each group were resuspended in 25 ml of NKM solution. The cell concentration was approximately 20 × 10^6 cells/ml and 2.5 ml were injected intraperitoneally into each rat. Tumor growth was evaluated 7 to 10 days later.

Results. Agglutination experiments. Of the sera from the two goats immunized with the 40S ribosomal subunits, one produced a marked agglutination of Novikoff hepatoma ascites cells (Fig. 1). The second produced much less agglutination and was not used further. Figure 1 also shows the comparative agglutination produced by antisera to the 40S and 60S ribosomal subunits. The results were quite similar, although the 40S antiserum used was more active in higher dilutions.

Although the antiserum to 60S subunits agglutinated Walker tumor ascites cells (1), the antiserum to the 40S subunits did not.

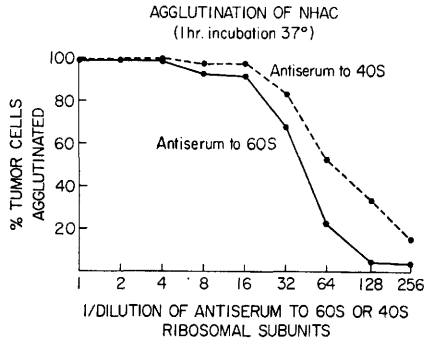

FIG. 1. Comparative agglutination of Novikoff hepatoma ascites cells by antiserum to tumor 60S ribosomal subunits and by antiserum to tumor 40S ribosomal subunits. The conditions for these incubations are described in the text.

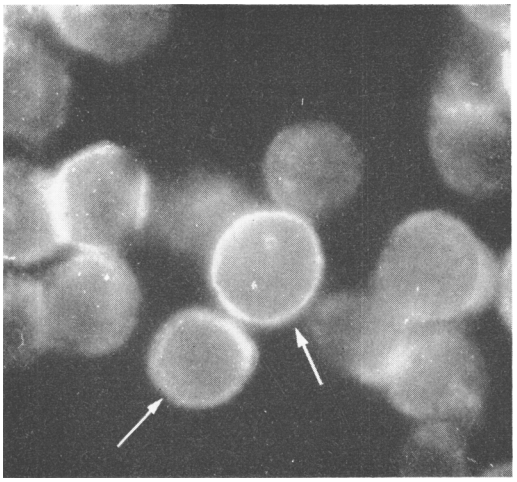

FIG. 2. Immunofluorescent stain of Novikoff hepatoma ascites cells. The cells were incubated for 1 hr at 37° with antiserum to 40S ribosomal subunits. The antiserum was removed and the cells were incubated in an equal volume of fluorescein-labeled rabbit antigoat γ-globulin for 1 hr at 37°. Fluorescent halos can be seen around the periphery of these cells but none were observed when normal goat serum was initially incubated with the cells.

Subsequent tests with the antiserum to the 60S ribosomal subunits failed to produce agglutination with the Walker tumor cells.[2] These antisera did not produce any greater agglutination of spleen white cells than normal goat serum.

Immunofluorescence. As was found earlier with the antiserum to the 60S subunits, a ring-type fluorescence on the cell surface was obtained with an indirect test in which the cells were incubated with 40S antiserum and then fluorescein-labeled rabbit antigoat γ-globulin. Cells incubated in normal goat serum and then fluorescein-labeled γ-globulin were not stained (Fig. 2).

Suppression of Tumor Growth. Figure 3 presents the results of experiments in which the Novikoff hepatoma ascites cells were incubated *in vitro* with the antisera to the 60S

[2] The agglutination of the Walker tumor cells by the antiserum to the 60S ribosomal subunits occurred with the sera obtained from the first and second bleeding of the goats. After each bleeding the antiserum activity decreased slightly and the antigen in the Walker tumor cells could no longer be agglutinated by the antiserum from the fourth bleeding.

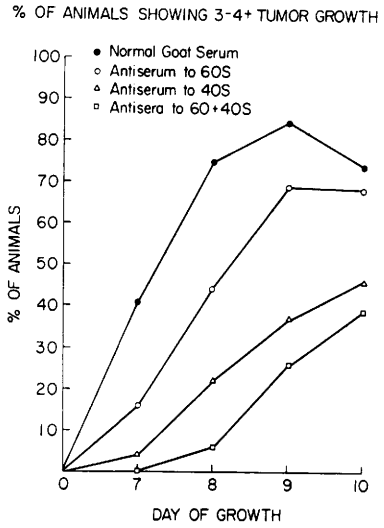

FIG. 3. In these experiments, the tumor cells were mixed with undiluted antiserum and incubated for 2 hr at 37°. For each group of 10 rats, 5×10^8 cells were incubated with either (A) 25 ml of normal serum, (B) 25 ml of antiserum to 60S subunits, (C) 25 ml of antiserum to 40S subunits or (D) a mixture of 12.5 ml of antiserum to 60S subunits and 12.5 ml of antiserum to 40S subunits. The cells were then pelleted at 400*g* for 3–4 min and resuspended in 25 ml NKM solution for injection into each group of ten rats. The cell suspension was injected into a total of 129 animals in five experiments.

and 40S subunits singly or in combination for 2 hr at 37°, resuspended in NKM and injected intraperitoneally into rats. Normal serum served as a control on growth results. The greatest delay in tumor growth was obtained when the combined antisera to both subunits were incubated with the cells. The growth suppression with the antiserum to the 40S ribosomal subunits was not as marked as with the combined sera, but it was greater than with the antiserum to the 60S ribosomal subunits. The data in Fig. 3 indicate that the effects of the antisera to the 40S and 60S subunits were additive. To determine whether doubling the amount of antiserum increased the inhibitory effect, the amount of antisera to 40S and 60S subunits was increased to 50 ml each for ten rats. Under these conditions, no further decrease in growth was found with the antiserum to the 40S subunits (Table I). On the other hand, doubling the amount of antiserum to the 60S subunits decreased growth to the same level as the smaller amount of antiserum to the 40S subunits. However, the combination of both antisera was more effective than either alone at both low and high concentrations, suggesting that the antisera may act separately or at least mainly at different sites.

Discussion. The present study demonstrates the presence of agglutinins for the NHAC in antisera of goats immunized with 40S ribosomal subunits of the tumor cells. The microscopic agglutination was similar with antisera from the 60S and 40S

TABLE I. Dosage Effects of Antisera on the Percentages of Animals with 3–4+ Tumor Growth.[a]

	Normal	Anti-60S	Anti-40S	Anti-60S + anti-40S
Single dose				
Day 7	41	14	3.5	0.0
Day 8	75	42	21	5.8
Double dose				
Day 7	33	0.0	0.0	0.0
Day 8	78	20	29	2.9

[a] In these experiments, tumor cells were mixed with undiluted antiserum and incubated for 2 hr at 37°. For each group of 10 rats, 5×10^8 cells were incubated with either: (A) 50 ml of normal serum, (B) 50 ml of antiserum to 60S subunits, (C) 50 ml of antiserum to 40S subunits, (D) a mixture of 25 ml of antiserum to 60S subunits and 25 ml of antiserum to 40S subunits. The cells were then pelleted at 400*g* for 3–4 min and resuspended in 25 ml of NKM solution for injection into each group of ten rats. The cell suspension was injected into a total of 59 animals in two separate experiments.

ribosomal subunits, although the antiserum for the 40S subunits had a slightly higher agglutination titer. Like the antisera to the 60S tumor ribosomal subunits, the antisera to the tumor 40S ribosomal subunits suppressed tumor growth and to a somewhat greater extent than the antisera to the 60S subunits. Neither of these antisera produced marked agglutination of spleen white cells and both produced a positive indirect immunofluorescence of tumor cells.

When the two antisera were combined, there was a greater suppression of tumor growth *in vivo* than with either antisera alone. This potentiation of growth inhibition was not primarily dose-related since twice the amount of either antiserum alone did not produce the same extent of growth suppression as the combination. The results suggest that at least two different antigens are responsible for the synergistic growth suppressive effects of these two types of antisera.

At least some of the inhibitory effects of these antisera are related to cell surface antigens since absorption experiments demonstrated almost complete removal of agglutinins with whole cells and weaker absorption with the ribosomal subunits. These results may reflect relative differences in concentrations of similar antigens of the ribosomes and the cell surfaces. In any event, different antigens are affected by antisera to the 40S and 60S subunits. Inasmuch as antisera to liver 60S ribosomal subunits also produced surface immunofluorescence, it would appear that there are ribosomal subunits closely juxtaposed to the plasma membrane as previously suggested by Warren (5).

Summary. Goats immunized with 40S ribosomal subunits of Novikoff hepatoma ascites cells produced antiserum containing agglutinins for Novikoff hepatoma ascites cells. The Novikoff hepatoma ascites cells gave a positive indirect fluorescence test. The antiserum to the 40S ribosomal subunits markedly inhibited the growth of the Novikoff hepatoma ascites cells. When antisera to 40S ribosomal subunits and the 60S ribosomal subunits were combined, the inhibition of growth was potentiated by comparison to the inhibition with either antiserum alone.

1. Busch, H., Busch, R. K., Spohn, W. H., Wikman, J., and Daskal, Y., Proc. Soc. Exp. Biol. Med. **137**, 1470 (1971).

2. Lamon, E. W., and Bennett, J. C., Proc. Soc. Exp. Biol. Med. **134**, 986 (1970).

3. Noll, F., and Bielka, H., Acta Biol. Med. Ger. **23**, 15 (1969).

4. Noll, F., and Bielka, H., Mol. Gen. Genet. **106**, 106 (1970).

5. Warren, L., Glick, M. C., and Nass, M. K., *in* "The Specificity of Cell Surfaces" (B. D. Davis and L. Warren, eds.), p. 109. Prentice-Hall, Englewood Cliffs (1967).

Received Mar. 6, 1972. P.S.E.B.M., 1972, Vol. 140.