Effect of β-3-Thienylalanine on Antibody Synthesis VII. Further Studies on the Inhibition of RNA Synthesis[1] (36152)

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Spleen cells from immunized rats represent a useful model to investigate the mechanism of action of β-3-thienylalanine (β-3-TA), as inhibitor of protein synthesis and cell multiplication (1), because of the well-documented new synthesis of RNA and protein and active cell division which occurs in these cells following antigenic stimulation (2). Recent work from our laboratory has described the inhibitory effect of β-3-TA on RNA and protein synthesis in immunized rat spleen cells (1). It was shown that, after an initial lag, a small burst of RNA synthesis occurs between 24 and 36 hr following immunization; but soon the synthetic activity declines to the levels of a nonimmunized, control rat spleen. The inhibition of RNA synthesis leads to cessation of protein synthesis after a short initial attempt, and this activity declines rapidly below the level observed in controls, nonimmunized animals. The work reported here was undertaken to determine which type of RNA was affected, since a preliminary experiment in which RNA was fractionated on a sucrose density gradient indicated that radioactive label in RNA from β-3-TA-treated immunized rat spleen cells accumulated in low molecular weight oligonucleotides. The studies of Mach and Vassalli (3, 4) provided a baseline to assess the types of RNA affected by β-3-TA treatment. These authors had described a sequence of appearance of RNA of different kinds in immunized animal spleen cells. We also employed various length pulses with labeled RNA precursors to distinguish between various species of RNA (5).

The results of these experiments seem to point out that β-3-TA depresses the synthesis of an RNA which, on the basis of labeling data, may be compatible with mRNA.

Materials and Methods. Sprague-Dawley male albino rats, weighing between 250 and 300 g, were used. Diets and immunization methods have been described elsewhere (6, 1). To avoid degradation of RNA by endogenous nucleases, liver supernant (LS) prepared as described previously, was employed in all experiments (7). At the time of sacrifice, the rat spleen was exposed under ether anesthesia, 300 μCi of ^{3}H-uridine (Schwarz Bioresearch, 20 Ci/mmole) were injected into the spleen and this organ was removed and immersed in ice-cold saline. The spleen was then homogenized in the presence of LS; and the homogenate was centrifuged at 4° and 12,000*g* for 15 min. The supernatant was collected, treated with a mixture of 20% Triton X-100 and 5% sodium deoxycholate, layered on a discontinuous sucrose gradient (1.38 over 2.0 *M*) in LS, and centrifuged at 14,800*g* for 4 hr at 4°. The polysomal pellet recovered was resuspended in 0.5% sodium dodecyl sulfate with 0.1 *M* NaCl in 0.01 *M* Tris to solubilize RNA. An aliquot of RNA was then examined by sucrose density gradient (5–20%) by centrifugation at 284,000*g* for 3.5 hr. The gradient was scanned at 260 mμ during collection of 0.5 ml fractions; and these were counted in a liquid scintillation counter.

Results. In a first series of experiments rats were sacrified at 0 time to obtain a base line of RNA synthesis in nonimmunized rat spleen cells and on days 2 and 6 after immu-

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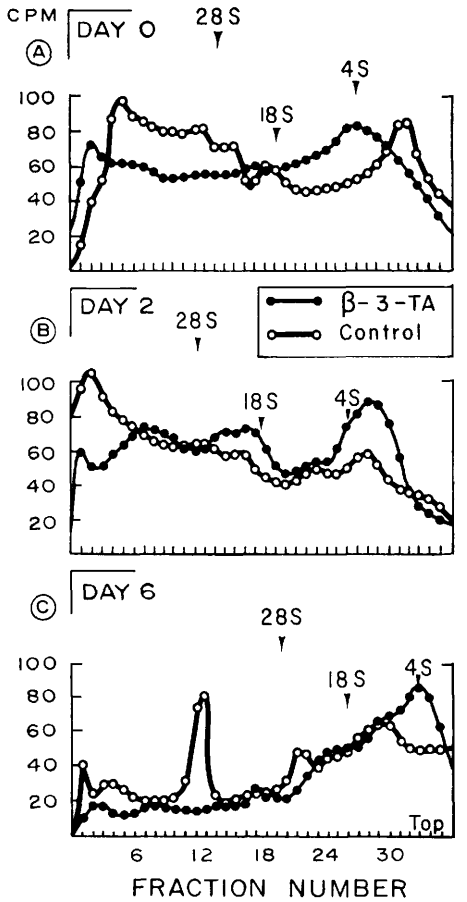

FIG. 1. Radioactivity accumulation in RNA fractionated on a sucrose density gradient as described in the text: On days 2 and 6, considerable differences exist between β-3-TA-treated and nontreated immunized animals which can be attributed to analogue administration. The position of the three main fractions of RNA are indicated by arrows.

nization with, and without, β-3-TA treatment, when RNA synthesis seems to be most drastically affected (1). ^{3}H-Uridine was given intrasplenically 3–5 min before sacrifice. Figure 1 illustrates the results obtained in a typical experiment of this kind. It is apparent (Fig. 1a) that newly synthesized RNA is quite heterogeneous in both β-3-TA-treated and nontreated spleen cells from the nonimmunized rat. At 2 days after immunization (Fig. 1b) in β-3-TA-treated immunized rats, there is a shift of labeled RNA to the lower molecular weight species migrating only slightly into the sucrose gradient. Some difference is also noted in the RNA migrating to the bottom of the gradient; RNA from non-β-3-TA-treated animals exhibits considerable accumulation of label which is not found in RNA from treated, immunized rat spleen cells. On day 6 (Fig. 1c) similar differences are found in RNA migrating at the top and bottom on the gradient; and, in addition, two peaks with considerable activity appear at approximately 30 S and 20 S in non-β-3-TA-treated immunized animals. In a second series of experiments rats were sacrificed 20 min after intrasplenic injection of ^{3}H-uridine and 0, 2, and 6 days after immunization. Both β-3-TA-treated and nontreated

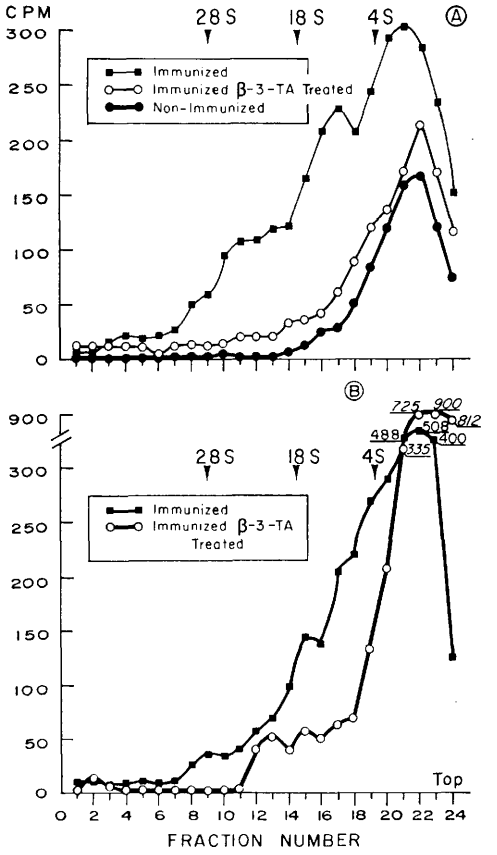

FIG. 2. Radioactivity accumulation in RNA separated on a sucrose density gradient as described in the text: A nonimmunized animal pattern is included in (A) for comparison with β-3-TA-treated and nontreated immunized animals. The effect of the analogue essentially prevents the changes due to immunization, at least at this time interval, as indicated by the almost superimposable curves.

rats were used at 2 and 6 days. Figure 2 summarizes the results of a typical experiment. The pattern of label here is different from that seen in the previous experiments, most likely because of the longer labeling time. A considerable difference was seen between treated and nontreated rats at both 2 and 6 days after immunization. In the treated rats (Fig. 2a), most of the activity is in RNA which accumulates at the top of the gradient; this reflects the pattern seen in control, nonimmunized, nontreated rats. RNA from the immunized rat spleen cells, on the contrary, shows considerable activity at the top of the gradient but also a definite peak at about 10 S, two definite shoulders at 16 to 25 S and two other shoulders at 28 S and at about 30 S. At 6 days after immunization (Fig. 2b) one finds a 16 S peak of radioactivity with a lower, not well-defined peak at 28 S in nontreated animals, while the treated exhibit two low peaks, one at 16 S and one at about 20 S. Again considerable amounts of radioactivity accumulate at the top of the gradient, particularly in the β-3-TA-treated rat spleen cell RNA.

Discussion. By fractionation on sucrose density gradient of ^{3}H-uridine labeled RNA extracted from spleen cells of immunized, β-3-TA-treated and nontreated rats, we have shown that β-3-TA treatment inhibits the synthesis of polyribosome-attached RNA produced during immune response to sheep erythrocytes. RNA synthesis does not seem to be affected in normal rats by β-3-TA treatment for 2 days, although a shift of activity from high to low molecular weight was seen. This shift is a phenomenon which is observed at all intervals in the first series of experiments and at 6 days in the second series and could represent either a block of RNA polymerase with accumulation of short chain RNA precursors by direct action on the polymerase itself or incorporation into this enzyme, which may thus be rendered partially inactive. It is interesting to note how the labeling pattern of the treated animals' RNA differs from that of the untreated. The former demonstrate marked accumulation of activity towards the lower molecular weight region and loss of activity in high molecular weight regions compared to the untreated animals. The mRNA of the length needed to code for light and heavy chains of immunoglobulin also seems to be affected. This would be expected to migrate to the regions of the gradient in which a peak is found in nontreated immunized rats 6 days after immunization. These two peaks are not present in β-3-TA-treated rat spleen cell RNA. While no definite conclusion can be made that we are measuring mRNA synthesis, the length of ^{3}H-uridine administration makes this likely (5).

These data then seem to further strengthen our previous findings that β-3-TA may inhibit antibody synthesis by depressing the synthesis of RNA, probably mRNA.

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