Defective RNA Synthesis in Lymphocytes from Patients with Primary Agammaglobulinemia

Abstract. *Addition of tetanus toxoid to sensitized lymphocytes from normal subjects and patients with "secondary" acquired agammaglobulinemia resulted in an increased incorporation of tritiated uridine into RNA and this increase was sustained for 48 to 72 hours in vitro. In contrast, the quantity of H^3-uridine incorporated into the RNA of lymphocytes from patients with "primary" acquired agammaglobulinemia decreased after 48 hours of exposure to specific antigen. Lymphocytes from patients with primary agammaglobulinemia were also distinguished by their subnormal and poorly sustained response to phytohemagglutinin and to rabbit antiserum to lymphocytes. These data suggest that the defect in primary agammaglobulinemia involves, either as a primary phenomenon or as a secondary event, an abnormality of quantitative RNA synthesis or of RNA stability in the circulating lymphocyte.*

"Acquired" agammaglobulinemia, not associated with malignancy of lymphoid tissues, like "congenital" agammaglobulinemia, is probably genetically determined (*1*). Although the precise defect in the production of immune globulins has not been defined, several hypotheses have been offered. Since plasma cells are absent from antibody-producing tissues in both congenital and acquired agammaglobulinemia, early investigators suggested that the defect in γ-globulin synthesis results from a genetic or acquired defect in a cell line or in the ability of lymphocytes to transform into plasma cells (*2*). As a result of observations of morphologic abnormalities in lymph nodes and thymus glands from patients with this disorder, the "cellular defect" hypothesis has been extended, and it has been suggested that a genetic abnormality exists in one or both components ("thymus-dependent" and "immune-globulin producing") of the antibody-producing system or in their integration (*3*). Since lymphocytes from patients with agammaglobulinemia are capable of morphologic transformation in response to certain stimuli but fail to produce γ-globulin, the defect may be the result of abnormal transcription of DNA with production of a quantitatively or qualitatively abnormal messenger RNA with concomitant impairment in the synthesis of the polypeptide chains of γ-globulin (see *4*).

Such speculations could logically be extended to include either an additional defect in DNA histone, which is expressed in lymphocytes but not in other somatic cells, or a defect in the process whereby messenger RNA is decoded and protein is synthesized. However, immunologically competent cells presumably have an afferent system by which the cells recognize an external stimulus to γ-globulin production, and an efferent system which begins with DNA transcription and terminates in the synthesis of γ-globulin. Such an afferent system is probably initiated by contact at the cell surface with foreign antigen or nonspecific stimulus or originates from material transferred to the lymphocytes from a carrier cell (*5*). There is no reason to assume a priori that the efferent rather than the afferent system is the site of the defective γ-globulin production in agammaglobulinemia.

In order to obtain additional information about the possible site or sites of the defect in γ-globulin synthesis, studies of RNA synthesis were undertaken with cultured lymphocytes from six normal subjects and seven subjects with "primary" or "secondary" agammaglobulinemia. Lymphocytes were obtained from heparinized venous blood and separated by nylon filters. Under the conditions described by Bach and Hirschhorn (*6*) for studies of morphologic transformation in response to antigen, cells (1×10^6 per milliliter) containing at least 90 to 95 percent lymphocytes were cultured 7 to 10 days after a "booster" dose of tetanus toxoid. Hemagglutination titers for the toxoid ranged between 1 : 10,000 and 1 : 40,000 in the normal subjects and were less than 1 : 640 in the agammaglobulinemic patients. After exposure of the cells to H^3-uridine (specific activity 2.97 c/mole, 0.3 μc/ml) for 0.5 to 72 hours, RNA was quantitatively precipitated with perchloric acid (*7*) or extracted with hot phenol (*8*). There was no qualitative difference in the patterns of labeling of phenol-extractable RNA in unstimulated normal or agammaglobulinemic lymphocytes or in the relative quantities of the isolated 28 and 18*S* ribosomal RNA and 4*S* transfer RNA in sucrose density gradients (Fig. 1). After a 30-minute exposure of cells to H^3-uridine, radioactivity was detectable in 4*S* RNA and in a heterogenous RNA component associated with DNA at the phenol-water interface (*p*H 9 fraction). After a 240-minute exposure of cells to H^3-uridine, radioactivity was also found in

Table 1. Incorporation of H^3-uridine into RNA by stimulated and unstimulated lymphocytes (control) after 48 and 72 hours of culture.

Immune globulins (% of normal)			H^3-uridine incorporated into RNA in unstimulated control cultures (count/min per 10^6 cells)*		Ratio of radioactive RNA in stimulated cultures to that in unstimulated cultures				Antiserum to lymphocyte§	
					Tetanus toxid†		Phytohemagglutinin‡			
γG	γA	γM	48 hr	72 hr	48 hr	72 hr	48 hr	72 hr	48 hr	72 hr
					Normal					
			3560	5160	1.99	4.40				
			3840	3520	0.93	1.26	17.2	33.5	2.21	3.21
			2640	2400	1.32	1.94			7.62	6.38
			2660	1780	1.33	4.54	39.7	28.8	15.1	4.47
			2790	1850	1.10	1.42	12.3	33.9	2.16	2.73
			2780	4160			34.6	45.4		
					"Secondary" acquired agammaglobulinemia					
16‖	<2	<2	1660	790	0.90	1.55	45.1	55.0	6.60	19.2
15¶	6	<2	2500	2250	1.94	1.45	54.8	127.0	3.14	14.5
					"Primary" acquired agammaglobulinemia					
18	<2	<2	1620	1040	0.95	0.33	11.6	17.6	1.44	0.55
7	<2	<2	1290	1080	0.68		6.45	7.95	1.21	0.60
11	<2	<2	1860	1890	1.22	0.23	5.74			
6	3	<2	2380	3640	1.25	0.26	30.8	18.8	7.10	2.88
<1.0	<2	<2	5200	4590			4.95	3.07	2.56	0.55

* All lymphocyte cultures were prepared in triplicate. † Eli Lilly 15 LF units/ml, dilution 1:1670. ‡Phytohemagglutinin-P, Difco, dilution 1:500. § Normal rabbit serum adsorbed with human erythrocytes produced no significant stimulation. ‖ Lymphoma. ¶ Thymoma.

28 and 18*S* ribosomal RNA. At this time, the specific activity of the *p*H 9 fraction had increased, but the pattern of distribution of radioactive RNA had not changed.

In control cultures of normal and agammaglobulinemic lymphocytes containing human serum albumin as the only additive, H^3-uridine was incorporated into acid-precipitable RNA over a 48- to 72-hour period. In most cultures, the maximum labeling of RNA occurred at about 48 hours (Table 1). In these cultures the decrease in labeled RNA between 48 and 72 hours was not the result of cell death or of exhaustion of labeled substrate. In four of five normal lymphocyte populations studied, exposure to tetanus toxoid was associated with increased incorporation of radioactive uridine into total acid-precipitable RNA at 48 hours; after 72 hours of exposure to antigen and H^3-uridine there was increased radioactive RNA in all of the normal lymphocyte cultures relative to nonstimulated control cultures. In two of four lymphocyte populations from patients with primary agammaglobulinemia there was increased labeling of RNA after a 48-hour exposure to tetanus toxoid; but in all three populations examined at 72 hours after exposure, there was a significant decrease, relative to controls, in labeled RNA in cultures exposed to antigen. Since supravital staining indicated more than 95 percent viability in both control and antigen-stimulated cultures, this decrease could not be attributed to cell death. Decrease in radioactive RNA may have resulted from excessive degradation of labeled RNA or failure to maintain RNA synthesis. Such a failure would quickly result in the loss of active RNA since the half-life of rapidly labeled RNA in lymphocytes is less than 2 hours (*9*).

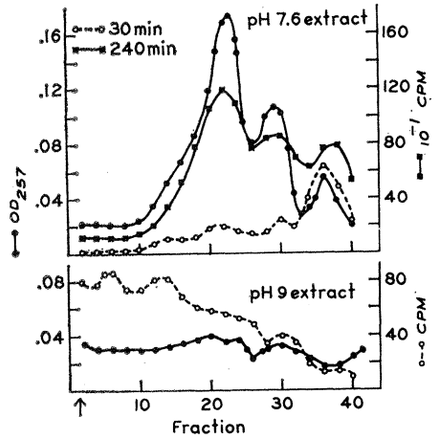

Fig. 1. Lymphocytes were labeled in vitro for 30 or 240 minutes with H^3-uridine prior to the isolation of RNA. Two RNA moieties were obtained in the phenol extraction; one extractable at 0°C and *p*H 7.6, the other at 60°C and *p*H 9. These fractions were subjected to centrifugation in sucrose gradients (5 to 20 percent) for 200 minutes at 100,000*g* (average). The optical-density profile of the fractions extracted at *p*H 7.6 obtained after exposure of 30 and 240 minutes to H^3-uridine were identical when equal quantities of RNA were placed on the gradient. The sedimentation values of the three optical-density peaks were 28, 18, and 4*S*, with the 28*S* peak migrating toward the bottom and with the 4*S* peak being at the top of the gradient. ●——●, Optical density at 257 mμ. ○---○, Count/min (CPM) in RNA isolated after 30 minutes' exposure to H^3-uridine. ■——■, Count/min in RNA isolated after 240 minutes' exposure to H^3-uridine.

The pattern of incorporation of H^3-uridine into RNA in response to antigen stimulation in lymphocytes obtained from two individuals with "secondary" acquired agammaglobulinemia was more consistent with the pattern in normal subjects. In all lymphocyte populations the response to tetanus was dependent on dose. At dilutions greater than 1 : 1670, the effect of antigen was progressively diminished; and at greater concentrations, it was magnified until toxic effects were observed in the range of 1 : 100 to 1 : 500.

Exposure to phytohemagglutinin was associated with a marked increment in RNA labeling by H^3-uridine in cultured lymphocytes from normal individuals and patients with agammaglobulinemia. A smaller increment in labeled RNA after 72 hours exposure to phytohemagglutinin was observed with lymphocytes obtained from patients with primary agammaglobulinemia (Table 1).

Rabbit antiserum to human lymphocytes when absorbed with human red cells also had a stimulatory effect on incorporation of H^3-uridine into RNA in lymphocytes. Again a difference was observed between the response of the normal and secondary agammaglobulinemic subjects and that of the primary agammaglobulinemic patients. In the latter group the response was poorly maintained after 48 hours and acid-precipitable radioactive RNA was lost from the cell (Table 1). The increased incorporation of uridine in normal lymphocytes was associated with increased RNA synthesis since total cellular RNA increased. The mechanisms of stimulation of RNA synthesis by antiserum to lymphocytes or by phytohemagglutinin are unknown. The obvious common denominator of these two agents is their ability to produce lymphocyte agglutination.

These data suggest that there is a difference between the lymphocytes of patients with primary and secondary agammaglobulinemia in their response to both nonspecific stimulation and specific antigens and reinforce the impression that the defect in primary agammaglobulinemia resides in the circulating lymphocyte. The quantitative abnormality in net RNA labeling by H^3-uridine, with subnormal and poorly sustained response to lymphocyte stimulants, is consistent with excessive ribonuclease activity which results in degradation of newly synthesized RNA or with a block somewhere between the afferent phase of the immune response and the initiation of globulin synthesis on the ribosome. It is possible, however, that the abnormality of incorporation of H^3-uridine is a secondary phenomenon and reflects some other defect in cell metabolism. For example, the RNA synthesis of both normal and agammaglobulinemic lymphocytes is sensitive to inhibitors of glycolytic enzymes (*9*). A direct toxic effect of antigen on a principal pathway of energy production is unlikely, however, in view of the sustained lymphocyte viability. Since the synthesis of ribosomal and soluble RNA appears to be grossly normal by the physical criteria used in these studies, it would seem more likely that the defect is associated with the afferent phase or with the transcription of DNA.

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References and Notes

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