PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 489–492 (1976)

Endotoxin-Stimulated Spleen Cells: Dissociation between DNA Synthesis and IgM Production at the Cellular Level[1] (39424)

BOBBY J. GORMUS[2] AND JOSEPH W. SHANDS, JR.

Department of Immunology and Medical Microbiology, University of Florida, College of Medicine, Gainesville, Florida 32610

Endotoxin (LPS) induces DNA synthesis and mitosis followed by IgM production in cultured murine B-lymphocytes (1–9). However, it appears that DNA synthesis is not a prerequisite for IgM production. Andersson and Melchers reported that hydroxyurea (Hu)-treated spleen cells responded to LPS with increased IgM synthesis while [^{3}H]thymidine incorporation was reduced more than 99% (10). This observation strongly supports a dissociation of DNA synthesis and IgM production, but to our knowledge a direct demonstration of this at the cellular level has not been reported. Using a method which permitted the simultaneous detection of cytoplasmic IgM and [^{3}H]thymidine incorporation in individual cells, we report here that inhibition of DNA synthesis by Hu does not result in an appreciable reduction in the percentage of cells synthesizing IgM. In addition, the cells making IgM have not incorporated [^{3}H]thymidine.

Materials and methods. Animals. C57Bl/6J Female mice were obtained from Jackson Labs, Bar Harbor, Maine.

Bacteria and endotoxin. LPS was extracted and purified from *S. typhimurium,* strain 7 (STM-7) by the phenol–water procedure (11).

Stimulation of spleen cells. Mouse spleen cells were prepared and cultured by the procedure of Alder *et al.* (12). Hydroxyurea, 10^{-2} *M*, when used, was added at the beginning of cultivation. Control cultures contained: (i) spleen cells plus Hu, (ii) spleen cells plus LPS, and (iii) spleen cells alone. One microcurie of [^{3}H]thymidine (Schwarz–Mann, Orangeburg, N.Y., 14.8 Ci/mmole) was added initially to each culture (2×10^6 spleen cells in 1 ml of RPMI-1640 containing 5% heat-inactivated normal human serum and antibiotics).

Fluorescent-autoradiographic procedure. Cells were removed from culture after 48 hr of LPS-stimulation and washed twice with RPMI-1640. Washed cells were deposited on microscope slides in a cytocentrifuge (Shandon Scientific Co., Sewickly, Pa.), fixed in ethanol for 1 min, and air-dried. Fluorescent antimouse IgM (fluorescein-conjugated heavy chain-specific, Meloy Laboratories, Springfield, Va.) was then layered over the cell pellet, and incubated at room temperature for 30 min. After washing with phosphate-buffered saline, preparations were either viewed directly on a fluorescence microscope or used in the combined fluorescent-autoradiography procedure (13). Best results were obtained by alternate dark field and fluorescence microscopy, utilizing a Leitz Ortholux microscope equipped with an Osram HBO 200 W light source, BG 38 and BG 12 excitors and a 470 or a 490 barrier filter.

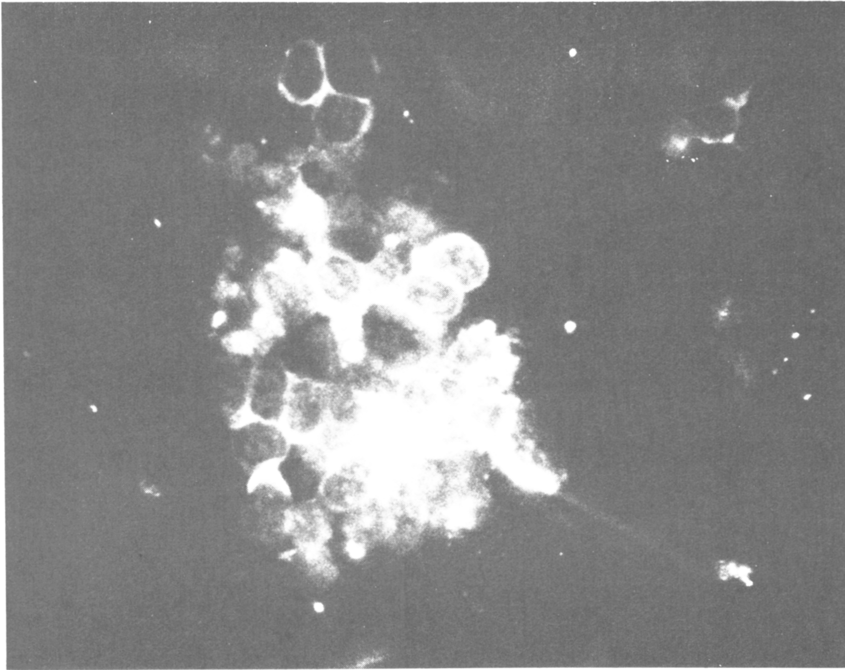
[^{3}H]thymidine incorporation. Triplicate determinations of [^{3}H]thymidine incorporation were made after 48 hr of LPS-stimulation by TCA precipitation of washed cells, followed by solubilization and scintillation counting (12).

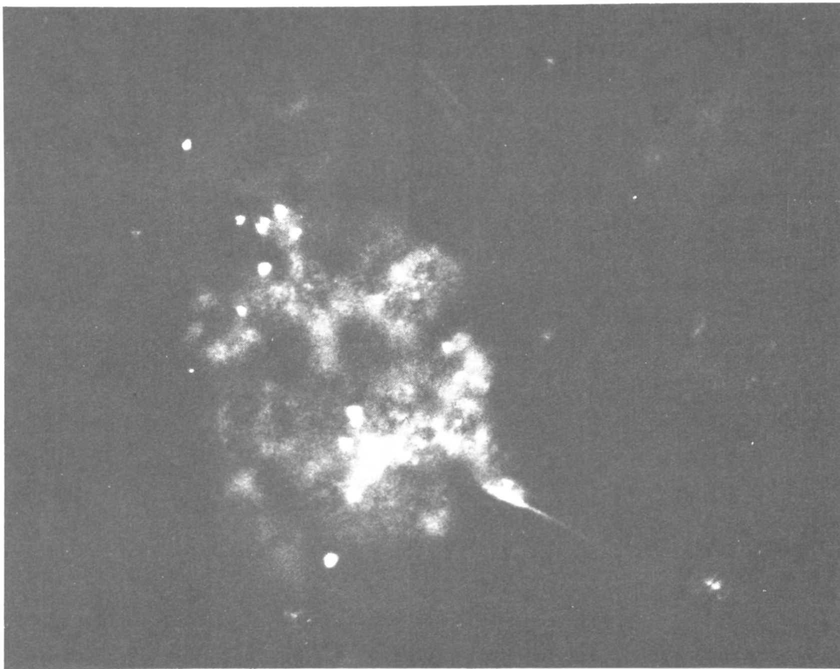
Autoradiography. Cytocentrifuged cells were treated with fluorescent anti-IgM and were dipped into Kodak NTB-3 emulsion (13). After optimal exposure, slides were developed, fixed, washed, dried and cover-slipped using phosphate-buffered saline-glycerine, 1:9. Cells were scored for fluorescence (cytoplasmic IgM) and [^{3}H]thymidine incorporation (grains over nuclei). This pro-

[1] This work was supported by Public Health Service Grants AI-07257 and 5TI-AI-0128 from the National Institutes of Allergy and Infectious Diseases.

[2] Supported by a postdoctoral fellowship from Smith-Kline and French Laboratories, Philadelphia, Pa. Present address: VA Hospital, 151, Research, 54th St. and 48th Ave. South, Minneapolis, Minn. 55417.

Copyright © 1976 by the Society for Experimental Biology and Medicine
All rights reserved.





FIGS. 1 and 2. Combined fluorescent autoradiography of LPS-stimulated spleen cells cultured with Hu; Fig. 1, focused on cells, Fig. 2, focused at the plane of the emulsion. ×648.

cedure permits the direct assessment of IgM content and [^{3}H]thymidine uptake by individual cells.

Results. The results in Table I show that the continuous presence of Hu in 48 hr LPS-stimulated cultures inhibited [^{3}H]thymidine incorporation more than 98%. Autoradiography showed that Hu blocked the uptake of [^{3}H]thymidine by cells which stained positively for cytoplasmic IgM (Figs. 1 and 2). On the other hand, spleen cells stimulated by LPS to produce IgM in the absence of Hu incorporated [^{3}H]thymidine (Fig. 3) in the previously reported fashion (13). It is clear, therefore, that at a cellular level LPS can induce IgM production by murine B cells in the absence of preceding DNA synthesis.

TABLE I. [^{3}H]THYMIDINE INCORPORATION BY LPS-STIMULATED CELLS IN THE PRESENCE OR ABSENCE OF HYDROXYUREA[a]

Exp.	CPM $\times 10^{-3}/2 \times 10^{6}$ cells $\pm$ SD		Percent reduction
	Control	10^{-2} *M* Hu	
1	143 ± 4.9	1.64 ± .32	98.9
2	242 ± 24.7	4.13 ± .51	98.3

[a] LPS added to conc of 10 μg/ml.

These observations confirm the report of Andersson and Melchers (10).

Surprisingly, the fluorescent antibody procedure showed that approximately 50% of the 48 hr stimulated spleen cells contained IgM whether or not Hu was present in the cultures. One would expect proliferation of the B cells to increase the percentage of IgM producers in cultures not inhibited by Hu. The observation is inexplicable at present. However, there are several possibilities, including dissimilar maturation to IgM production and restriction of cell proliferation by [^{3}H]thymidine of high specific activity.

Summary. LPS stimulation of mouse spleen cells in the presence of Hu resulted in almost total suppression of [^{3}H]thymidine incorporation without affecting the percentage of cells induced to produce IgM. Utilizing a method which permitted the simultaneous measurement of IgM production and [^{3}H]thymidine incorporation in individual cells, it was demonstrated directly that LPS stimulation of IgM production can occur in the absence of DNA synthesis.

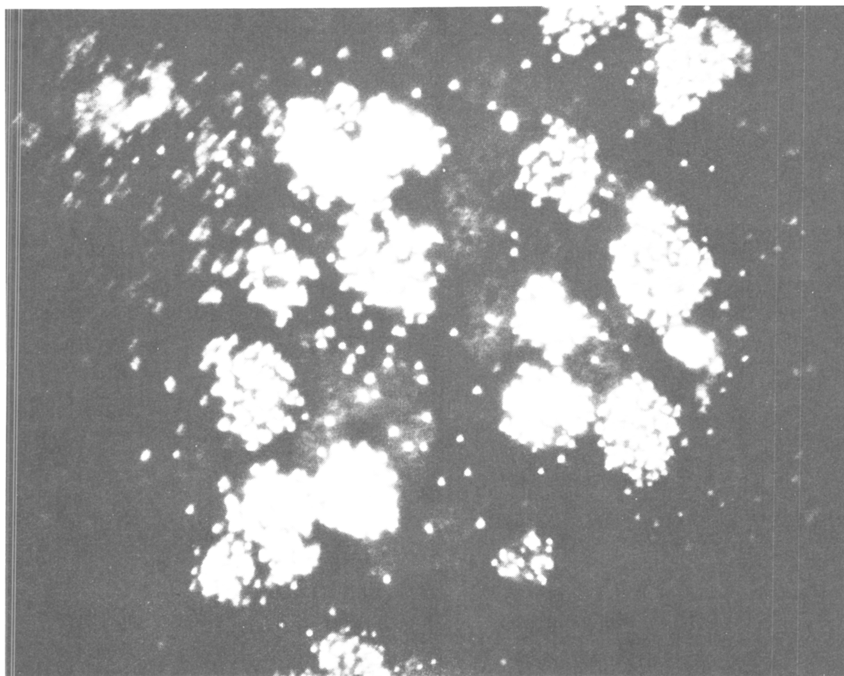


FIG. 3. Same procedure used on LPS-stimulated spleen cells cultured without Hu. The photograph is focused at the plane of the emulsion. ×648.

1. Andersson, J., Möller, G., and Sjöberg, O., Cell. Immunol. **4,** 381 (1972).
2. Gery, I., Krüger, J., and Spiesel, S. J., J. Immunol. **108,** 1088 (1972).
3. Andersson, J., Sjöberg, O., and Möller, G., Transplant. Rev. **11,** 131 (1972).
4. Andersson, J., Sjöberg, O., and Möller, G., Eur. J. Immunol. **2,** 349 (1972).
5. Shands, J. W., Jr., Peavy, D. L., and Smith, R. T., Amer. J. Pathol. **70,** 1 (1973).
6. Gormus, B. J., Crandall, R. B., and Shands, J. W., Jr., J. Immunol. **112,** 770 (1974).
7. Melchers, F., and Andersson, J., Transplant. Rev. **14,** 76 (1973).
8. Melchers, F., and Andersson, J., Eur. J. Immunol. **4,** 181 (1974).
9. Melchers, F., and Andersson, J., Eur. J. Immunol. **4,** 687 (1974).
10. Andersson, J., and Melchers, F., Eur. J. Immunol. **4,** 533 (1974).
11. Westphal, O., Lüderitz, O., and Bister, F., Z. Naturforsch. **7b,** 148 (1952).
12. Adler, W. H., Takiguchi, T., Marsh, B., and Smith, R. T., J. Exp. Med. **131,** 1049 (1970).
13. Gormus, B. J., and Shands, J. W., Jr., J. Immunol. **115,** 118 (1975).

Received March 18, 1976. P.S.E.B.M. 1976. Vol. 152.