HUMORAL IMMUNOSTIMULATION

I. Increased Uptake of [^{125}I]Iododeoxyuridine and [^{3}H]Thymidine into TNP-Cells Treated with Anti-TNP Antibody*

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Evidence to support an immunostimulation theory of tumor growth has recently been reviewed by Prehn (1, 2). Basically the immunostimulation theory proposes that both humoral and cellular immune responses can, under certain conditions, facilitate tumor growth by direct effect upon the tumor cells. Enhancement of tumor cell growth, on the other hand, is thought to be due to an enhancing antibody which acts by blocking cell-mediated immunity and thus indirectly facilitates tumor cell growth (3). The immunity effect proposed by Prehn is a biphasic one; that is, mild immune response to tumor cells accelerates tumor cell growth, whereas a strong immune response is cytotoxic (1, 2, 4).

There is a large body of literature on the ability of the lymphocyte to undergo blast transformation with a concomitant increase in DNA synthesis upon exposure to plant lectins, bacterial mitogens, and antilymphocyte antibody. Sell and Gell have shown that there is blast transformation and stimulation of protein, RNA, and DNA synthesis when rabbit lymphocytes are exposed to antibody directed to their allotypic surface immunoglobulins (5–7). Apart from experiments designed to demonstrate complement-mediated cytotoxicity, there have been relatively few reports of the effect of antibodies on the growth of nonlymphocytic cells in vitro. Adverse effects of antibodies on cell metabolism and growth have been observed even in the absence of complement, as exemplified in a recent study in a mouse leukemic cell (8). HeLa cells, a human cell line, were agglutinated by antisera prepared in heterologous species, but there was no effect upon tritiated thymidine and uridine uptake as judged by autoradiographic studies after relatively short time intervals (9). Another group demonstrated a two-fold increase in the phospholipid content of HeLa cells exposed to anti-HeLa antibodies and complement 8–24 h previously, but this was felt to be

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due to a repair process initiated by immune damage of cell membranes rather than a direct stimulation of membrane metabolism (10). In the one report of apparent enhancement of cellular function by antibody, active transport of potassium was shown to be increased in sheep LK red blood cells after incubation with sheep anti-L antibody (11). This communication describes an antibody-mediated stimulation of radioactive nucleoside incorporation (and presumably of DNA synthesis) by tumor cell lines maintained in tissue culture. Preliminary reports of this work have appeared (12, 13).

Materials and Methods

Cell lines:—Hela, HEp-2, and mouse L cells were purchased commercially (Grand Island Biological Co., Grand Island, N. Y., and American Type Culture Collection, Rockville, Md.) and maintained in nutrient medium (NM)[1] which consisted of Eagle's minimum essential medium (MEM) plus 10% (v/v) fetal calf serum (FCS). Penicillin G (100 U/ml) and streptomycin (100 μg/ml) were included in the NM. The cells were plated in 250 ml T-flasks (Falcon Plastics, Div. of BioQuest, Oxnard, Ca.) and incubated in a humidified 5% CO_2 atmosphere at 37°C. Media of cells were changed three times a week. When the cells became confluent (about 10^6 cells/ml), they were subjected to a 2 min trypsinization (0.05% trypsin) at 37°C in Ca^{++}, Mg^{++}-free Hanks' buffered salt solution (Ca^{++}, Mg^{++}-free HBSS), scraped up with a rubber policeman, agitated into single-cell suspension, and replated in fresh media at 10^5 cells/ml in a total volume of 10 ml per flask. The cell lines were monitored periodically for mycoplasma through the courtesy of Dr. Carl Harford, Department of Medicine, Washington University School of Medicine.

Antibody:—Antibodies specific for the 2,4,6-trinitrophenyl (TNP) determinant, obtained by hyperimmunization of randomly bred albino rabbits with TNP-labeled bovine gamma globulin in complete Freund's adjuvant, were specifically purified according to Eisen and Little (14). The antibody was at least 92% pure as determined by quantitative precipitin analysis with TNP-bovine serum albumin; it had an average association constant for ϵ-TNP lysine of about 5×10^7 L/M at 25°C as determined by fluorescence quenching (14). The protein concentration of antibody solutions was determined from the absorbancy at 280 mM (1 cm, 1% = 15.0).

Antibody Conjugate.—Conjugates of purified anti-TNP antibody and glucose oxidase (Boehringer Mannheim Corp., New York) were prepared at high protein concentrations in the presence of a bifunctional reagent (diethylmalonimidate) as previously described (15). The glucose oxidase activity in the conjugates was determined by the method of Keston and Brandt (16).

TNP-Substitution of Cells.—A single-cell suspension was obtained from cell monolayers by digestion with 0.05% trypsin for 2 min as described above. Optimal cell densities of the original flasks were $1\text{–}2 \times 10^5$/ml. The cells were washed twice with NM, three times with HBSS, and resuspended in 1.95 ml HBSS. 50 μl of a freshly prepared solution of TNP sulfonic acid (TNP-SO_3H) solution in HBSS (2 mg/ml) was added to the cell suspension to a final (TNP-SO_3H) concentration of 50 μg/ml. The reaction was carried out for 20 min at 37°C in 5%

[1] *Abbreviations used in this paper:* Ca^{++}, Mg^{++} free HBSS, calcium, magnesium free HBSS; DNP, 2,4-dinitrophenyl; FCS, fetal calf serum; HBSS, Hanks' buffered salt solution, hNM, nutrient medium containing heat inactivated FCS; MEM, Eagle's minimal essential medium; NM, nutrient medium; PBS, 0.15 M NaCl, 0.01 M phosphate, pH 7.4; TCA, trichloroacetic acid; [^{3}H]TdR; [^{3}H]methyl-thymidine; TNP, 2,4,6-trinitrophenyl; TNP-SO_3H, trinitrophenyl sulfonic acid; [^{125}I]UdR, [^{125}I]-5 iodo-2′-deoxyuridine.

CO_2 with continuous agitation (180 cycles/min), maintaining the tubes at a 45° angle. The reaction was stopped by the addition of 8 ml of NM and the TNP-cells were washed three times with NM. All centrifugations were made at 400 *g* for 2 min. HeLa and HEp-2 cells treated in this fashion were shown to contain 3.5 and 6.0 × 10^9 TNP molecules per cell, respectively (17). Control cells were handled in an identical fashion with the omission of TNP-SO_3H.

Treatment of Cells with Antibody.—Control cells and TNP-cells were washed three times in 0.15 M NaCl, 0.01 M phosphate, pH 7.4 (PBS) or NM with 10% heat-inactivated FCS (hNM); they were resuspended in 1 ml of either of these media at a cell density of 0.5 to 2 × 10^6 cells/ml. Small volumes (10–50 μl) of antibody-enzyme conjugate or purified anti-TNP antibody were added to bring the final antibody concentration to 0.01–150 μg/ml. In some conditions ε-DNP-lysine (10^{-4} M final concentration) was added before the addition of antibody. In most experiments the cells were preincubated with antibody, antibody-enzyme conjugate, or control IgG for 30 min at 37°C. Unbound antibody-enzyme conjugate or purified anti-TNP antibody was then removed by centrifugation and by washing the cells three times with hNM. The antibody-treated cells were incubated in hNM in 12 × 75 mm tissue culture tubes for periods of up to 4 days at initial cell densities of 0.25–3 × 10^5 cells/ml. Some tubes contained lactoperoxidase (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 50 μg/ml and sodium iodide at 2 × 10^{-5} M. Nucleoside uptake by cells was measured with [^{125}I[5 iodo-2′-deoxyuridine ([^{125}I]UdR) or [^{3}H]methyl-thymidine ([^{3}H]TdR).

Radioactivity Measurements.—[^{125}I]UdR (Amersham, Searle, Arlington Heights, Ill., original sp act 200–2000 mCi/mmol) was added at various times after the initiation of the culture. In some experiments a small volume of [^{125}I]UdR-containing medium was added directly to the cell cultures. In other experiments the cells were centrifuged, the old medium aspirated, and fresh medium containing [^{125}I]UdR added to the cell pellets. The range of final radioactivity concentration was 0.025–1.0 μCi/ml. The period of incubation with [^{125}I]UdR was 24 h. Retained radioactivity was determined by washing the cells twice with NM (centrifugation 1000 *g* for 5 min) followed by measurement of gamma emission in a well type scintillation counter. Recounts of retained radioactivity were made in some experiments after two extractions with 6% trichloroacetic acid (TCA). [^{3}H]TdR was obtained from New England Nuclear (Boston, Mass.) at an original sp act of 6.7 Ci/mmol; it was aded to the culture tubes in an identical fashion to the [^{125}I]UdR using a final radioactivity level of 0.5 μCi/ml. At the end of the 24-h period the contents of the tubes were rinsed onto a cell harvesting manifold, washed twice with cold 6% TCA, dried on the filter paper, digested with tissue solubilizer (NCS, Amersham, Searle Corp., Arlington Heights, Ill.), dissolved in toluene scintillator, and counted by liquid scintillation.

RESULTS

Consistent stimulation of incorporation of [^{125}I]UdR into TNP cells treated with the antibody-glucose oxidase conjugate alone was noted. Representative data obtained with TNP-substituted HEp-2 cells are presented in Table I. As described in detail elsewhere (15) the combination of lactoperoxidase (50 μg/ml), iodide (2 × 10^{-5} M), and antibody-enzyme conjugate was cytotoxic as seen by the reduction of CPM of ^{125}I incorporated and by the reduction of cell counts in microwells. Despite the stimulation of [^{125}I]UdR uptake by the antibody conjugate, there was no increase in cell counts in microwells. However, the incubation conditions differed in that the microwell experiments were terminated after 24 h, whereas the nucleoside uptake experiments were continued for 48 h. In-

TABLE I

*TNP-Substituted HEP-2 Cells Treated with Glucose Oxidase Anti-TNP Antibody Conjugate, Lactoperoxidase and Iodide: [^{125}I]Idodeoxyuridine Uptake and Microcytotoxicity Data**

Conditions	CPM of ^{125}I in cell pellets‡	Stimulation ratio (experimental/control)	Cell counts in micro-wells§	Stimulation ratio (experimental/control)
1. TNP-HEP-2 cells (100,000/ml)	46,780 ±1158	—	218 ±13	—
2. TNP-HEP-2 cells and antibody conjugate (11 μg/ml)	101,968 ±1572	2.18 ($P \pm 0.001$)	203 ±16	0.93 ($P > 0.5$)
3. TNP-HEP-2 cells, antibody conjugate, lactoperoxidase (50 μg/ml), and iodide (2×10^{-5} M)	12,564 ±393	0.27 ($P < 0.001$)	40 ±5	0.18 ($P < 0.001$)

* TNP cells were incubated in PBS with and without anti-TNP-glucose oxidase conjugate for 30 min, washed once with PBS, three times with hNM, incubated for 24 h in hNM with and without lactoperoxidase and iodide in 12 × 75 mm tubes at a cell density of 100,000/ml; they were then centrifuged at 400 *g* for 3 min, and cultured in hNM containing [^{125}I]UdR 0.6 μCi/ml for an additional 24 h. The cells were harvested by centrifugation, washed twice in hNM, and counted. 20 μl aliquots of the cultures of conditions 1–3 were cultured in microwells for 24 h, fixed in calcium-formalin, stained with Giemsa, and counted in a microscope using a standard grid.

‡ Expressed as mean of triplicate determinations ± standard error of mean (SEM).

§ Expressed as mean of six determinations ±SEM.

cubation of microwell test plates for longer than 24 h caused drying of the plates and inaccurate cell counts.

That the coupling of glucose oxidase to the purified anti-TNP antibody did not, in some nonspecific fashion, cause enhanced nucleoside uptake can be seen in Fig. 1. Purified anti-TNP antibody itself stimulated the incorporation of radioactive nucleoside. The effect of the antibody appeared to be dose-related in this experiment although a full range of antibody concentrations was not covered.

The stimulation of [^{125}I]UdR uptake by antibody exhibited a characteristic time dependency (Fig. 2). In TNP-cells the ratio of [^{125}I]UdR uptake in antibody-treated to nonantibody-treated cells was maximal 24–72 h after exposure to antibody. The same time course of stimulation was observed regardless of whether the cells were exposed briefly to anti-TNP antibody for 30 min and then washed or whether the cells were cultured in the presence of anti-TNP antibody. It is apparent that as the time of incubation progresses only those cells treated with larger amounts of antibody continue to show significant stimulation of [^{125}I]UdR uptake, suggesting a consumption of antibody with time or a changing antibody dose reponse curve. While the absolute uptake of [^{125}I]UdR over a 24-

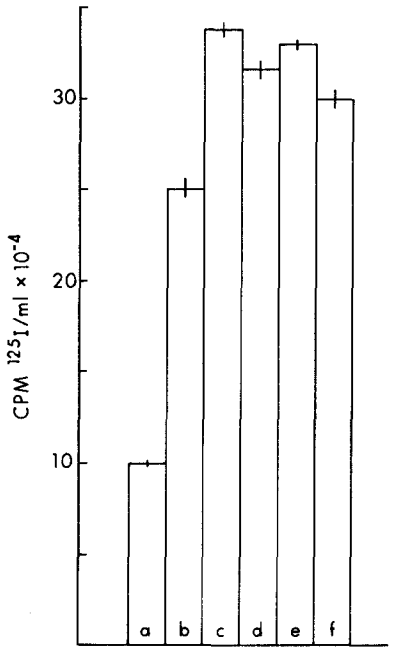

FIG. 1. Stimulation of [^{125}I]UdR uptake in TNP-substituted HEp-2 cells treated with anti-TNP antibody: Effect of the antibody dose on the level of stimulation. TNP-cells (see text for preparation) were exposed to anti-TNP antibody at various concentrations in PBS for 30 min, washed three times with hNM, and cultured for 24 h in 12 × 75-mm test tubes at an initial cell density of 120,000/ml; the cells were then centrifuged at 400 *g* for 3 min and cultured for another 24 h in hNM containing [^{125}I]UdR at 1.0 μCi/ml; after incubation with [^{125}I]UdR the cells were washed two times in hNM, counted, washed twice in 6% TCA, and counted again. CPM in hNM and 6% TCA were within 2% of each other. Columns: (*a*) control; (*b*) 1.0 μg/ml anti-TNP antibody; (*c*) 5.0 μg/ml anti-TNP antibody; (*d*) 10 μg/ml anti-TNP antibody; (*e*) 50 μg/ml anti-TNP antibody; (*f*) 100 μg/ml anti-TNP antibody. Results are expressed as means of triplicate determinations ± SE of mean represented as bars above and below the top of the columns. Differences between the control and experimental values are statistically significant at the $P < 0.001$ level of confidence.

h period declines as the age of the cultures increases, the difference between control and antibody-treated cells is still highly significant statistically, e.g., $P < 0.001$ for differences between *a* and *c*, *d* in Fig. 2 *B*. This experiment also shows that anti-TNP antibody stimulates nucleoside uptake in TNP-HeLa cells as well as TNP-HEp-2 cells. The data for day 2 in 2 *A* do not show an unequivocal stimulation pattern; however, by day 3 the differences between control and antibody-treated cells had become meaningful (see legend to Fig. 2 *A*). Evidence is presented below for stimulation of [^{125}I]UdR uptake in a third cell line, mouse L cells (Table III).

The duration of trypsinization influenced the ability of TNP-cells to be stimulated by anti-TNP antibody (Table II). Cells merely scraped off mono-

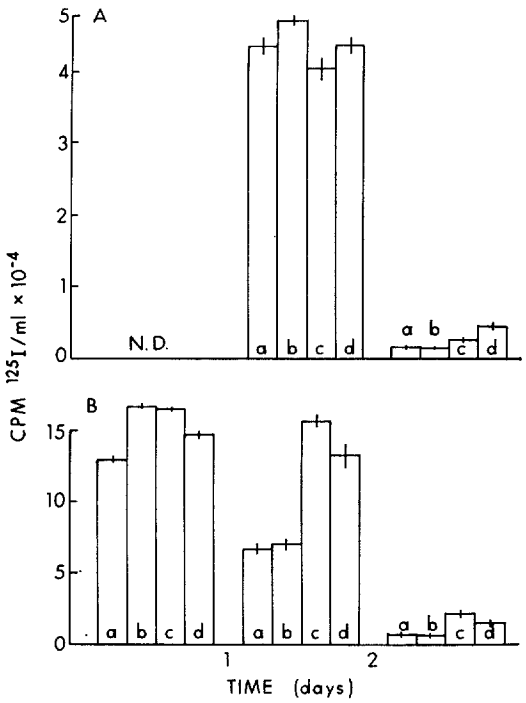

FIG. 2. (*A*) Stimulation of [^{125}I]UdR uptake in TNP-HeLa cells after exposure to anti-TNP antibody for 30 min: effects of time interval after initial antibody exposure on level of stimulation. Cells were treated in the same manner as described in Fig. 1. Initial cell density was 85,000/ml. Cells were labeled with [^{125}I]UdR 1 μCi/ml from 24–48 and 48–72 h after initial antibody exposure. (*a*) control; (*b*(0.1 μg/ml anti-TNP antibody; (*c*) 1.0 μg/ml anti-TNP antibody; (*d*) 10.0 μg/ml anti-TNP antibody. Bars above and below columns represent SE of the mean. Differences between *a* and *c*, *d* on day 3 were significant at the $P < 0.02$, and the $P < 0.01$ level of confidence, respectively (absolute values: (*a*) 1,755 $\pm$ 69, (*c*) 2,455 $\pm$ 173, (*d*) 4,383 $\pm$ 311). Other differences between control and experimental values were not statistically significant. (*B*). Stimulation of ^{125}IUdR uptake in TNP-HEp-2 cells during continuous exposure to medium containing anti-TNP antibody for 72 h: effect of time interval after initial antibody exposure on level of stimulation. Cells were treated as described in the legend to Fig. 1 with the exceptions that the anti-TNP antibody was added in hNM rather than PBS, the cells were not washed after exposure to antibody, and the [^{125}I]UdR was added in a small supplemental volume of hNM. Initial cell density was 60,000/ml. (*a*) control; (*b*) 0.1 μg/ml anti-TNP antibody; (*c*) 1.0 μg/ml anti-TNP-antibody; (*d*) 10.0 μg/ml anti-TNP antibody. Cells were labelled with [^{125}I]UdR, 1 μCi/ml, for 0–24, 24–48, 48–72 h after initial antibody exposure. Bars above and below columns represent SE of the mean. Differences between *a* and *b*, *c* for day 1, *a* and *c* for day 2, *a* and *c*, *d* for day 3 are statistically significant at the $P < 0.001$ level of confidence, while the differences between *a* and *d* for day 1, *a* and *d* for day 2 are statistically significant at the $P < 0.01$ level of confidence. Pertinent values for day 3 were (*a*) 8,375 $\pm$ 189, (*c*) 20,916 $\pm$ 485, (*d*) 15,746 $\pm$ 380.

TABLE II

*Stimulation of [^{125}I]Iododecxyuridine Uptake in TNP-Substituted HEP-2 Cells Treated with Anti-TNP Antibody: Effects of Trypsinization on Maximal Stimulation**

Conditions	No trypsinization		2-min trypsinization		5-min trypsinization	
	CPM in cell pellets‡	Stimulation ratio (experimental/control)	CPM in cell pellets‡	Stimulation ratio (experimental/control)	CPM in cell pellets‡	Stimulation ratio (experimental/control)
1. TNP-HEP-2 cells (80,000/ml)	504 ±33	—	31,098 ±1968	—	43,065 ±2042	—
2. TNP-HEP-2 cells and ANTI-TNP (1.5 μg/ml)	834 ±31	1.65 ($P < 0.01$)	48,102 ±1856	1.54 ($P < 0.01$)	42,977 ±3637	1.00 ($P > 0.9$)

* Cells were treated as described in Table I with the following exceptions: The length of trypsinization was 0, 2, and 5 min at a trypsin concentration of 0.05% Cells were pulsed with [^{125}I]UdR, 0.027 μCi/ml, from 48 to 72 h after initial exposure to antibody (30 min).

‡ Expressed at mean of triplicate determinations ±SEM.

layers and those digested with trypsin 0.05% for 2 min could be stimulated after TNP substitution, but cells digested with 0.05% trypsin for 5 min or longer were not. Pellets of TNP-cells from 2-min trypsinization preparations were yellow, whereas those from the 5 min or longer preparations were white, thus suggesting that extensive trypsinization removed a protein or glycopeptide component of the cell membrane to which TNP-groups could attach. It is uncertain whether the effect of extensive trypsinization is on the cell surface antigen concentration per se or whether residues critical for stimulation by anti-TNP antibody are being removed. Trypsinization itself caused a large increase in the ability of the cells to incorporate nucleoside, a phenomenon previously recognized (18).

Three lines of evidence that stimulation of radioactive nucleoside uptake in TNP-cells is immunologically mediated are presented in Table III: (*a*) anti-TNP antibody had no effect on unsubstituted L cells in marked contrast to its effect on TNP-substituted L cells. (*b*) 100 μM ε-DNP lysine, a known hapten inhibitor of anti-TNP antibody, completely inhibited the stimulation phenomenon while having no apparent deleterious effect itself on the cells. (*c*) "Normal" rabbit gamma globulin at the same protein concentration as the anti-TNP antibody produced very little increase in [^{125}I]UdR incorporation in TNP-cells. Indeed, several subsequent experiments, including data in Fig. 3, showed that normal rabbit gamma globulin did not cause any increase whatsoever in incorporation of radioactive nucleoside.

Simultaneous, identical comparisons of the antibody-stimulated cellular uptake of [^{125}I]UdR and [^{3}H]TdR are seen in Fig. 3. There was a striking parallel in the relative degree of stimulation of incorporation of ^{125}I and [^{3}H]CPM into the cells. Thus the validity of using [^{125}I]UdR as a measure of DNA synthesis was confirmed. In this experiment the incorporation of nucleoside was almost

TABLE III

*Stimulation of [^{125}I]Iododeoxyuridine Uptake in TNP-Substituted L Cells Treated with Anti-TNP Antibody: Specificity of Reaction**

Conditions	CPM in cell pellets‡	Stimulation ratio (experimental/control)
A group		
L cells (47,000/ml)	1,948 ± 93	—
L cells and anti-TNP (15 μg/ml)	1,694 ± 269	0.93 ($P > 0.40$)
L cells, anti-TNP, and DNP-lysine (10^{-4}M)	1,554 ± 115	0.80 ($P > 0.10$)
L cells and rabbit γG (14 μg/ml)	2,103 ± 483	1.04 ($P > 0.70$)
B group		
TNP-L cells (35,000/ml)	686 ± 79	—
TNP-L cells and anti-TNP (15 μg/ml)	11,310 ± 546	16.9 ($P < 0.01$)
TNP-L cells, anti-TNP, and DNP-lysine (10^{-4}M)	991 ± 60	1.50 ($P > 0.10$)
TNP-L cells and rabbit γG (14 μg/ml)	948 ± 14	1.42 ($P < 0.05$)

* Cells were treated as described in Table I with the following exceptions: The A group of cells was not reacted with TNP-sulfonic acid; ε-DNP lysine was added to some cells just prior to exposure to antibody (30 min); normal rabbit gamma globulin was added to some cells at the same protein concentration as purified anti-TNP antibody; cells were pulsed with [^{125}I]UdR at 0.025 μCi/ml 48–72 h after exposure to antibody.

‡ Expressed as mean of duplicate determinations ± SEM.

identical in the cells exposed to antibody for only 30 min or throughout the time period of culture (72 h).

Kinetic studies of [^{125}I]UdR uptake (Fig. 4) showed that radioactive nucleoside entered antibody-treated TNP-L-cells faster than cells treated with equivalent amounts of normal rabbit gamma globulin. The greater rate of incorporation of nucleoside in the presence of antibody persisted for at least 1 h. Table IV shows that at 60 min almost all of the radioactivity that had entered the cells during the preceding hour was precipitable with TCA indicating that very little had remained in the soluble nucleoside pool.

DISCUSSION

In the present study anti-TNP antibodies have been shown to alter nucleoside transport in TNP-substituted cells in tissue culture. Similar responses to antibodies in other cell systems may have been missed because of problems in interpretation created by nonspecific serum effects on cell growth. We chose to use the TNP system for our initial studies because of the availability of a method for obtaining highly purified antibody and a means of sensitizing cells with a chemically defined hapten without interfering with cell viability. The specificity controls available with the TNP system leave no doubt that the stimulation of nucleoside incorporation in TNP-substituted tumor cell lines after exposure to

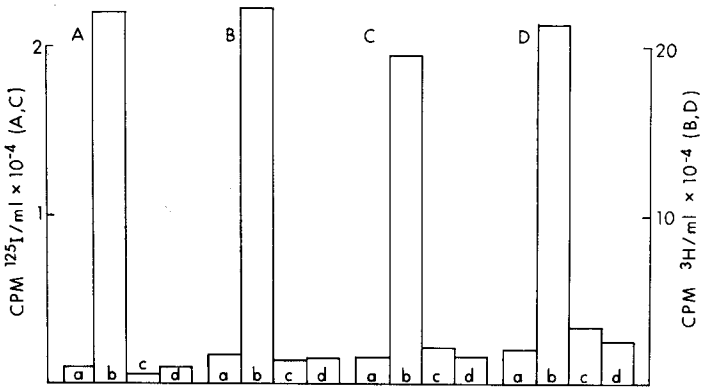

FIG. 3. TNP-substituted L cells treated with anti-TNP antibody: Comparison of stimulation of [^{125}I]UdR and [^{3}H]TdR uptake. Cells were treated as described in Table I and III. (*A*) [^{125}I]UdR uptake of TNP-cells treated with anti-TNP antibody for 30 min. (*B*) [^{3}H]TdR uptake of TNP-cells treated with anti-TNP antibody for 30 min; (*C*) [^{125}I]UdR uptake of TNP-cells cultured in hNM with anti-TNP antibody for 72 h; (*D*) [^{3}H]TdR uptake of TNP-cells cultured in hNM containing anti-TNP antibody for 72 h. Radioactivity concentrations were 0.04 μCi/ml for [^{125}I]UdR and 0.30 μCi/ml for [^{3}H]TdR. Cells were labeled with radioactive nucleoside 48–72 h after initial antibody exposure. Original cell density was 50,000/ml. (*A* and *B*) and 40,000/ml (*C* and *D*). Conditions: (*a*) control; (*b*) anti-TNP antibody (7 μg/ml); (*c*) anti-TNP antibody (7 μg/ml) and ϵ-DNP lysine (10^{-4} M); (*d*) rabbit gamma globulin (7 μg/ml). Values are expressed as mean of triplicate determinations. Differences between *a* and *b* (*A–D*) were statistically significant at a $P < 0.001$ level of confidence, between *a* and *c* (*C* and *D*) at a $P < 0.05$, $P < 0.01$ level of confidence, respectively, but differences between *a* and other values were not significant.

anti-TNP antibody is immunologically mediated: (*a*) The stimulation is obtained with microgram quantities of highly purified rabbit anti-TNP antibody, and equivalent amount of rabbit gamma globulin do not stimulate. (b) Three different cell lines (HeLa, HEp-2, and L) from two different species show stimulation of nucleoside incorporation when TNP groups are present, whereas unsubstituted cells give no hint of stimulation. (*c*) The degree of stimulation of nucleoside incorporation is dependent on the dose of anti-TNP antibody. (*d*) 100 μM ϵ-DNP lysine, a known hapten inhibitor of TNP-anti-TNP reactions, completely abrogates the stimulatory effect. (*e*) A delayed stimulation of nucleoside uptake occurs when TNP-cells are exposed to anti-TNP antibody for only 30 min and then carefully washed as well as when the antibody is left in the medium. (*f*) Prolonged trypsinization of cells renders them unable to react with antibody and display enhanced incorporation of nucleoside, presumably due to removal of functional groups capable of forming covalent bonds with TNP. It is thus quite clear that anti-TNP antibody can attach to surfaces of TNP-cells and cause these cells to incorporate increased quantities of nucleosides, an effect which is maximal several days after initial exposure to antibody.

What physiologic significance this observation holds is not clear from the data

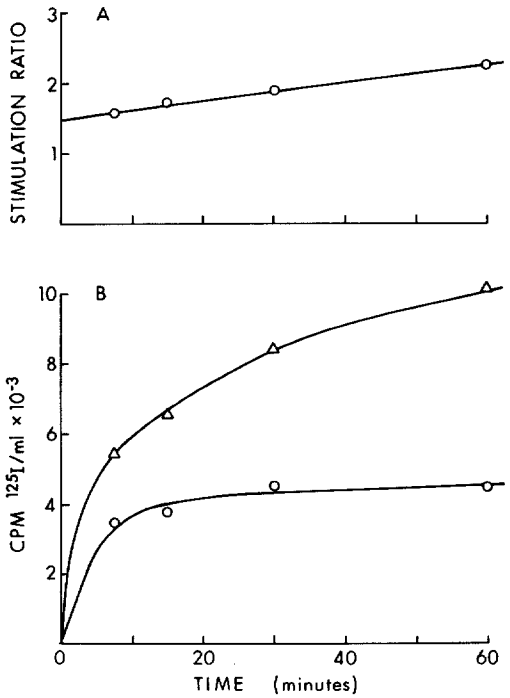

FIG. 4. TNP-substituted L cells treated with anti-TNP antibody: a kinetic analysis of [^{125}I]UdR incorporation into cells 48 h after exposure to antibody. Cells were treated as described in Tables I–III. (*A*) Ratio of incorporation of [^{125}I]UdR by TNP-cells treated with anti-TNP compared to those treated with rabbit gamma globulin. (*B*) Rate of incorporation of [^{125}I]UdR by TNP-cells treated with anti-TNP antibody and rabbit gamma globulin. Cells were exposed to antibody and rabbit gamma globulin for 30 min, plated at 30,000 cells/ml, and incubated in hNM for 48 h. [^{125}I]UdR at a final activity of 0.40 μCi/ml was added to triplicate tubes of each condition for 7.5, 15, 30, and 60 min. Cells were centrifuged at 1000 *g* for 1 min, washed twice with hNM, and counted. Triangles are TNP-cells treated with 7 μg of anti-TNP antibody and circles are TNP-cells treated with 7 μg of rabbit gamma globulin. Results are expressed as the mean of triplicate determinations. Results are statistically significant at the $P < 0.01$ (7.5 min and 30 min) and $P < 0.001$ (15 min and 40 min) levels of confidence.

presented and from our inability to detect meaningful and consistent differences of cell numbers or total cellular DNA between experimental and control cells (data not shown). While we took precautions to exclude complement from our system, the possibility exists that complement secreted by the cells themselves damages cell membranes (as a nonlethal effect) in the presence of the TNP-anti-TNP reaction and thus initiates membrane repair with subsequent increased DNA synthesis. It is more probable that a perturbation of cell surfaces (TNP-cells) occurs upon combination with anti-TNP antibody, producing a direct stimulation of cell membrane function which causes an intense increase in radio-

TABLE IV

*Percent of CPM of ^{125}I Precipitable with 6% TCA in TNP-L-Cells Treated with Anti-TNP Antibody and Rabbit Gamma Globulin**

Conditions	Time of incubation of cells with [^{125}I]UdR			
	7.5 min	15 min	30 min	60 min
TNP-L-cells (30,000/ml) and rabbit γG (7 μg/ml)	60.8	72.4	84.8	97.7
TNP-L-cells and anti-TNP (7 μg/ml)	63.7	75.7	86.0	95.8

* Cells were treated as described in Table I. At the end of 48 h of incubation [^{125}I]UdR was added to experimental and control cells for various time periods. Cells were spun at 1,000 *g* for 1 min, washed with 1 ml of hNM twice, counted in a gamma counter, washed with 1 ml of 6% TCA twice, and counted again.

active nucleoside uptake and DNA synthesis. A well-established precedent for similar immune stimulation of lymphocytes exists (5–7). In lymphocytes phytolectins also cause a very large increase in cellular incorporation of DNA precursors and DNA synthesis with little or no change in total cell numbers or DNA content (19). To carry the parallel further the greatest increase in nucleoside transport and DNA synthesis in lectin stimulated cells is between 48 and 72 h (7), corresponding to the period in which anti-TNP antibody stimulation of nucleoside uptake is maximal in TNP cells. Whether the other metaboic changes seen in lectin-stimulated cells are produced by anti-TNP antibody remains to be investigated.

Despite the unique advantages of the TNP system in terms of specificity, in other respects the use of hapten-substituted cells is not ideal. The cellular antigen is diluted as cells enlarge and divide; because of the artificial nature of the cell membrane TNP antigen, it is uncertain whether the information obtained can be extrapolated to immune phenomena involving natural cell antigens. However, we have been able to obtain similar metabolic responses as well as considerable increases in cell number with antibodies to natural cell antigens (20) as will be discussed in detail in a future paper.[2]

SUMMARY

Interaction of microgram quantities of highly purified rabbit anti-TNP antibodies with TNP-substituted HeLa, HEp-2, and L cells caused an intense stimulation of radioactive nucleoside ([^{125}I]UdR and [^{3}H]TdR) uptake which was maximal 24–72 h after exposure of cells to antibody. The stimulation of nucleoside uptake and presumaly DNA synthesis was shown to be immuno logically mediated because unsubstituted cells were not stimulated by anti-TNP

[2] Shearer, W. T., G. W. Philpott, and C. W. Parker. 1973. Humoral Immunostimulation. II. Increased cell growth of and increased nucleoside incorporation into HeLa, HEp-2, L, and MOPC-315 cell lines treated with respective anticell antibodies. Manuscript in preparation.

antibody, normal rabbit gamma globulin did not stimulate TNP-cells, and a hapten inhibitor, ε-DNP-lysine, prevented the stimulation of TNP-cells by anti-TNP antibody. These findings demonstrate that interaction of antibody with cell surface antigen can alter cell membrane transport, and possibly can enhance cell growth.

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