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Transferrin Receptor Induction Is Required for Human B-Lymphocyte Activation but not for Immunoglobulin Secretion

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Transferrin receptors are expressed on proliferating cells and are required for their growth. Transferrin receptors can be detected after, but not before, mitogenic stimulation of normal peripheral blood T and B cells. In the experiments reported here we have examined the regulation of transferrin receptor expression on activated human B cells and whether or not these receptors are necessary for activation to occur. Activation was assessed by studying both proliferation and immunoglobulin secretion. We have determined that transferrin receptor expression on B cells is regulated by a factor contained in supernatants of mitogen-stimulated T cells (probably B-cell growth factor). This expression is required for proliferation to occur, since antibody to transferrin receptor (42/6) blocks B-cell proliferation. Induction of immunoglobulin secretion, however, although dependent on PHA-treated T-cell supernatant, is not dependent on transferrin receptor expression and can occur in mitogen-stimulated cells whose proliferation has been blocked by antitransferrin receptor antibody. In addition, we have demonstrated that IgM messenger RNA induction following mitogen stimulation is unaffected by antitransferrin receptor antibody. These findings support a model for B-cell activation in which mitogen (or antigen) delivers two concurrent but distinct signals to B cells: one, dependent on B-cell growth factor and transferrin receptor expression, for proliferation, and a second, dependent on T cell-derived factors and not requiring transferrin receptors, which leads to immunoglobulin secretion.

Key words: transferrin receptors, B-cell growth factor, proliferation, immunoglobulin synthesis

Transferrin receptors are expressed on all proliferating cells in vivo and in vitro and appear to be essential for cell growth [1]. Peripheral blood lymphoid cells do not normally express detectable amounts of this receptor [2–4], although transferrin itself is a major serum glycoprotein. Transferrin receptors, however, can be readily de-

Abbreviations used: BCGF, B-cell growth factor; CSA, Cowan 1 Staphylococcus aureus; PHA, phytohemagglutinin; PBMCs, peripheral blood mononuclear cells; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline.

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tected on T cells following mitogenic stimulation [5]. This expression is not only a phenotypic characteristic of activated T cells but a functional requirement of the activation process as well [5], and furthermore appears to be regulated by T cell-specific growth factor (interleukin-2) [5].

Regulation of B-cell proliferation is less well understood than that of T cells, in part because specific growth factors for B cells have only recently been identified [6–12]. On the other hand, B-cell function, ie, immunoglobulin secretion, has been well characterized. For many years, regulation of B-cell differentiation has been an area of interest and controversy and it remains unclear whether B-cell proliferation is required for differentiation to occur or the two events are independent of each other [12–18].

The purpose of this study was twofold: 1) to determine what regulates transferrin receptor expression on activated B cells and to determine the importance of these receptors for proliferation and 2) to examine whether proliferation is required for differentiation in B cells by making use of monoclonal antibodies that block the transferrin receptor. Our results show that 1) transferrin receptor expression is dependent on prior exposure to B-cell growth factor-containing supernatant, 2) B-lymphocyte proliferation is dependent on transferrin receptor expression even in the presence of BCGF-containing medium, and 3) proliferation may not be required for secretion.

MATERIALS AND METHODS

Additions to Cells

CSA (Cowan I Staphylococcus aureus cells; Calbiochem-Behring Corp., La Jolla, CA) was added at a final dilution of 1:100,000 at the beginning of each experiment. Culture supernatant containing B-cell growth factor (BCGF) activity as well as T-cell replacing factor (TRF) activity was prepared as follows. Briefly, T cells were cultured in RPMI 1640 containing 10% fetal calf serum (GIBCO, Grand Island, NY) for 3 days in the presence of 2.5 μg/ml phytohemagglutinin (PHA, Wellcome Research Labs, Beckenham, England). Cell-free supernatant was stored at 4°C until use. It was added at a final concentration of 10% (v/v). Before using this supernatant (designated as *PHA supernatant*), it was screened by ELISA (see below) to determine if it contained detectable amounts of immunoglobulin. Only those PHA supernatants that were negative were used in these experiments. Mouse monoclonal antibody to the transferrin binding site of the human transferrin receptor (42/6; a gift of Dr. Ian Trowbridge, Scripps Clinic, La Jolla, CA) was added at a final concentration of 5 μg/ml at the start of each experiment (in sterile phosphate-buffered saline, azide-free).

Lymphocyte Isolation and Fractionation

Fresh peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood from normal donors by differential density centrifugation with lymphocyte separation media (LSM, Litton Bionetics, Kensington, MD). T cell-enriched and B cell-monocyte populations were obtained using anti-$F(ab)_2$ immunoabsorbent affinity columns as previously described [19] with the modification that cells adherent to the columns were eluted by mechanical agitation of the column matrix rather than by addition of α-globulin. PBMCs were placed on the immunoabsorbent

columns and a nonadherent cell fraction (T cell-enriched) was collected in a first eluate. The column was then washed extensively with media and mechanically disrupted to dislodge adherent B cells and monocytes; the latter were collected in a second eluate. The T-cell fraction contained 85% sheep red blood cell (SRBC) rosetting cells, less than 1% surface immunoglobulin staining cells, and less than 1% nonspecific esterase staining cells. The column-adherent B-cell monocyte fraction contained 45% surface immunoglobulin staining cells, 40% nonspecific esterase staining cells, and less than 1% SRBC rosetting cells.

Preparations of strictly T cell-depleted fractions were obtained by treating the column-adherent fraction with anti-Lyt-3 monoclonal antibody (1.0 μg/10^6 lymphocytes) (New England Nuclear, Boston, MA) and complement (Low-Tox-H Rabbit Complement, Cedarlane Laboratories, Hornby, Ontario, Canada). After treatment, flow cytometric (FACS) analysis could detect no residual T cells in this preparation (see below). Pure B-cell preparations were obtained by passing the column-adherent fraction over Sephadex G-10 columns. Macrophage-depleted B cells were collected in the eluate, which was then subjected to anti-Lyt-3 and complement treatment as before. There were no residual T cells or macrophages in this preparation that could be detected by FACS analysis (see below).

Cell Purity Determination

Cell purity was determined by staining with several mouse monoclonal antibodies (B1: pan-B cell, Coulter Electronics, Hialeah, FL; LYT3: pan-T cell, New England Nuclear; OKM1: monocytes, Ortho Diagnostics, Westwood, MA), followed by fluorescein-conjugated goat antimouse IgG (Tago, Burlingame, CA) and assayed by flow cytometric analysis using a FACS II (Becton-Dickinson, Sunnyvale, CA) [5]. Cells were stained with nonimmune mouse ascites (BRL, Gaithersburg, MD) followed by FITC-anti-IgG as a negative control.

Culture Methods

Cell cultures for proliferation and immunoglobulin synthesis were established in identical fashion. Cells were cultured (10^5-well; 5×10^5/ml) in 96-well flat bottom microtiter plates (Flow Laboratories, Inc., McLean, VA). Culture media consisted of RPMI 1640 (GIBCO) supplemented with 10% heat-inactivated fetal calf serum (FCS, GIBCO), 2 mM glutamine, 25 mM HEPES, 100 U/ml penicillin, and 100 μg/ml streptomycin. Each well contained 0.2 ml. In appropriate cultures culture media were supplemented with 10% PHA culture supernatant. After various times cells were washed and counted using a Coulter Model ZF cell counter (Coulter Electronics, Hialeah, FL). In some experiments cells were resuspended in phosphate-buffered saline (PBS) containing 2% albumin and 0.2% azide for FACS analysis. Culture supernatants were removed and made cell free by centrifugation and IgM levels were determined by ELISA as described below.

ELISA Determination

Secreted human IgM was determined using the following procedure. Culture supernatant (100 μl) was added to 100 μl of phosphate-buffered saline (PBS) (containing 0.05% Tween-80) in a 96-well microtiter plate (Nunc Immunoplate I, GIBCO), which had been pretreated with 200 μl of goat antihuman IgM (heavy-chain specific; 1.6 μg/ml in PBS; Tago, Inc.) for at least 24 hr. After at least 3 hr, the plates were

washed and 200 μl biotinylated goat antihuman IgM (1:500 dilution in PBS/Tween buffer; Tago, Inc.) was added. After 1 hr, plates were washed and 200 μl of ABC reagent (1:100 dilution in 0.05 M Tris HCl, pH 7.6; Vector Labs, Burlingame, CA) was added. After 30 min, plates were washed and 200 μl of OPD solution (5 mg O-phenylenediamine (Aldrich Chemical Co., St. Louis, MO), 500 μl methanol, 125 μl H_2O_2, and 50 ml H_2O) was added to develop color. The reaction was stopped by adding 50 μl 8 N H_2SO_4 to the wells. Plates were read on a Titertek Multiskan (Flow Labs) and optical densities (absorbance at 492 nm) of unknowns were compared to those of a standard curve of human IgM added to each plate. The method is sensitive to an IgM level of 10 ng/ml.

Determination of Transferrin Receptors

42/6 is directed against the transferrin binding site of the transferrin receptor [20] but does not interfere with the binding of another monoclonal antibody, OKT9 (Ortho Diagnostics, Westwood, MA), which recognizes the transferrin receptor but does not block transferrin binding [15]. Thus, cells can be stained with OKT9 in the presence of 42/6 and then reacted with fluoresceinated goat antimouse IgG (Kirkegaard and Perry, Gaithersburg, MD). This antibody does not recognize 42/6 (an IgA) and so background staining is minimal. Transferrin receptor-positive cells are then determined by FACS analysis as previously described [5].

Measurement of Immunoglobulin Messenger RNA

The relative abundance of IgM messenger RNA (Cu mRNA) per cell was assayed by the cytoplasmic dot blot method of White and Bancroft [21] as previously described by Cossman et al [22]. A ^{32}P nick-translated genomic DNA probe of the constant region of the μ-chain gene (Cu) [23] was hybridized to the dot blots, autoradiograms were developed, and relative amounts of specific RNA were compared by scanning densitometry.

Detection of Cytoplasmic Immunoglobulin

Cytoplasmic IgM was detected by direct immunofluorescence of cytocentrifuge preparations as previously described [24].

RESULTS

The conditions required for transferrin receptor induction on B cells were examined (Fig. 1A). Incubation of B cell-enriched (T-depleted) fractions with both CSA and PHA supernatant resulted in transferrin receptor expression on B cells. The percentage of receptor-bearing cells increased to nearly 80% by the seventh day of culture. Addition of antibody to the transferrin receptor (42/6, 5 μg/ml) at the beginning of culture completely inhibited the expression of transferrin receptors during the course of the experiment. Incubation of the cells with CSA alone resulted in no significant transferrin receptor expression.

B-cell thymidine incorporation was determined after 7 days of culture in the presence of CSA alone; CSA plus PHA supernatant; or CSA, PHA supernatant, and 42/6 (Fig. 1B). CSA alone resulted in no significant incorporation above background, whereas incubation with CSA and supernatant for 7 days resulted in a 25-fold stimulation of thymidine incorporation. The presence of 42/6 in the culture medium

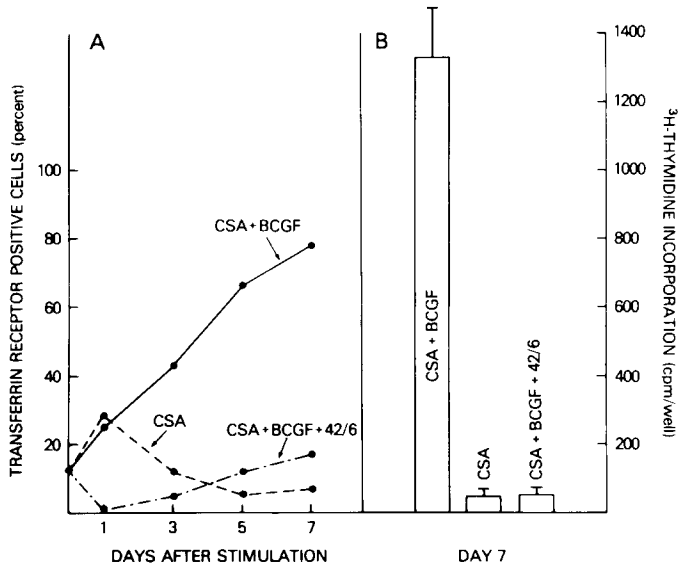

Fig. 1. B cell-enriched populations (T cell-depleted) were cultured in the presence of CSA and PHA supernatant; CSA alone; or CSA, PHA supernatant, and 42/6 (antitransferrin receptor antibody, 5 μg/ml). On various days after the beginning of the cultures, percentage of transferrin receptor-positive cells was determined by FACS analysis (A) and ^{3}H-thymidine incorporation was determined following a 4-hr pulse with 1 μCi ^{3}H-thymidine (B).

completely blocked this incorporation (Fig. 1B). Thus, thymidine incorporation was correlated to transferrin receptor expression in that those conditions that promoted the expression of receptor-stimulated thymidine incorporation, while conditions inhibiting transferrin receptor expression inhibited thymidine incorporation.

To further demonstrate the close association between transferrin receptor expression and thymidine incorporation by B cells, we examined the transferrin receptor number per cell (fluorescence intensity) at various times following addition of CSA and PHA supernatant. We also examined thymidine incorporation at the same time points. Thymidine incorporation was only stimulated by CSA plus supernatant and not by CSA alone or CSA, supernatant, and 42/6 (Fig. 2B). Incorporation reached a peak on day 3 and then declined for the remainder of the experiment. Transferrin receptor number per cell (Fig. 2A) also reached a peak on day 3 and then declined to a value on day 7 still about twice that of control cells. The temporal correlation between these two variables (transferrin receptor number per cell and amount of thymidine incorporated) is quite striking. Furthermore, conditions that did not permit transferrin receptor expression blocked thymidine incorporation over the course of the experiment. Thus, PHA supernatant only stimulated thymidine incorporation by B cells when these cells possessed detectable transferrin receptors.

We performed cell counts on B cells cultured with various agents for 5 days (Table I). CSA alone or supernatant alone caused no significant proliferation, whereas addition of PHA supernatant to CSA-containing cultures (on day 0) resulted in a greater than threefold proliferation by day 5. Addition of 42/6 to these cultures completely inhibited this proliferation. Addition of another monoclonal antibody,

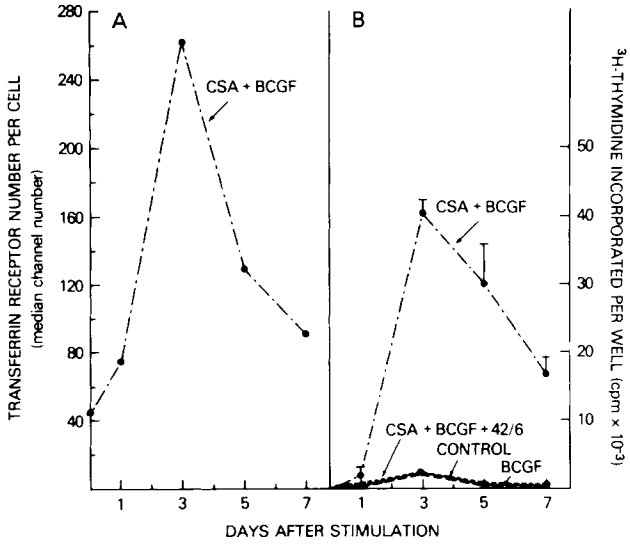

Fig. 2. B cell-enriched populations (T cell-depleted) were cultured alone, or in the presence of CSA; CSA and PHA supernatant; or CSA, PHA supernatant, and 42/6(●). Transferrin receptor number per cell was determined calculating the median fluorescence intensity of the positively stained cells at various times after stimulation, using FACS analysis (A). The FACS was standardized each day with fluorescent beads (Polysciences, Warrenton, PA) so that fluorescent intensities obtained on different days could be compared. ^{3}H-thymidine incorporation was determined at each time point as described in Figure 1 (B).

TABLE I. Antitransferrin Receptor Antibody Inhibits B-Cell Proliferation

Additions[a]	Cell number[b]
—	4.55 ± 0.36
CSA	5.70 ± 0.19
BCGF/SUP	5.21 ± 0.78
CSA + BCGF/SUP	15.57 ± 3.25
CSA + BCGF/SUP + 42/6	3.36 ± 0.99

[a]CSA (final concentration 1:100,000), BCGF-containing supernatant (final concentration 1:10, v/v), and 42/6 (final concentration 5 μg/ml) were added at the start of the experiment.
[b]Cells were set up at 5 × 10^5/ml and counted on day 5 using a Coulter model ZF cell counter. Cell numbers are expressed as cells × 10^{-5} per ml ± SD.

OKT10 (Ortho Diagnostics) (10 μg/ml) recognizing an antigen that activated B cells also express, had no effect on transferrin receptor expression, thymidine incorporation, or cell proliferation (data not shown).

Addition of 42/6 at the initiation of culture did not prevent the blastogenesis induced by CSA and PHA supernatant when this parameter was measured on day 5, suggesting that perhaps blockade of the transferrin receptor was not affecting B-cell maturation. To test this we looked at the ability of 42/6 to affect immunoglobulin secretion induced by CSA and PHA supernatant.

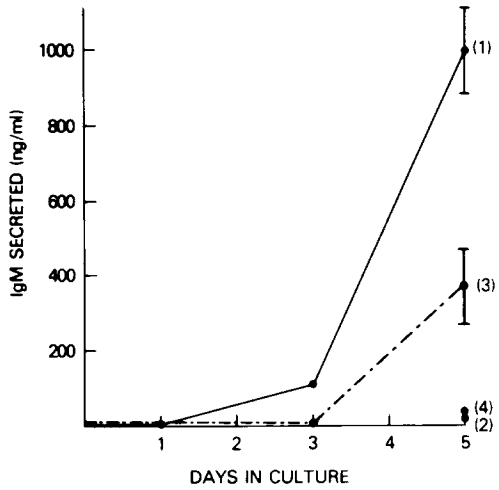

Fig. 3. B cell-enriched populations (T cell-depleted) were cultured with CSA and PHA supernatant (1), nothing added (2), CSA, PHA supernatant, and 42/6(3), or CSA alone (4). IgM secretion was measured by ELISHA at various times after stimulation.

A time course of IgM secretion was generated over a 5-day period (Fig. 3). In the presence of CSA and supernatant, IgM secretion was detectable by the third day of culture and increased greatly by day 5. Culture with CSA alone induced no IgM secretion by day 5, whereas addition of 42/6 to B cells cultured with CSA and supernatant markedly reduced the amount of IgM secreted per ml.

Although B cells cultured with CSA and PHA supernatant together with 42/6 secreted markedly less IgM than a control population untreated with 42/6 (Fig. 3), the discrepancy observed could be due to the decreased number of cells in the antitransferrin receptor-treated wells. To investigate this possibility we treated a purified B-cell population [depleted of monocytes ($<1\%$) and T cells ($<4\%$)] with various reagents and measured cell number per well and IgM secreted per well on day 5 (Fig. 4).

B cells were seeded at 500,000 per ml and proliferated only in the presence of CSA and PHA supernatant. Incubation with CSA alone or addition of 42/6 to cultures containing CSA and supernatant completely inhibited B-cell proliferation (Fig. 4A). When IgM secretion was measured and expressed as amount secreted per well 42/6 markedly reduced secretion (Fig. 4B), but when the amount secreted was expressed per 10^5 cells present on day 5 addition of antitransferrin receptor antibody to cultures had no effect on IgM secretion (Fig. 4C).

These results suggest that B cells incapable of proliferation may still respond to differentiating signals following antigen or mitogen exposure. To shed more light on this possibility, we examined the time course of specific IgM mRNA induction following CSA stimulation of a B-cell population. The results can be seen in Figure 5. IgM mRNA was quantified on a daily basis for 5 days after mitogen stimulation and the largest induction was found 1 day after initiation of the culture. IgM mRNA remained elevated for 4 days and returned nearly to control levels by the fifth day of culture.

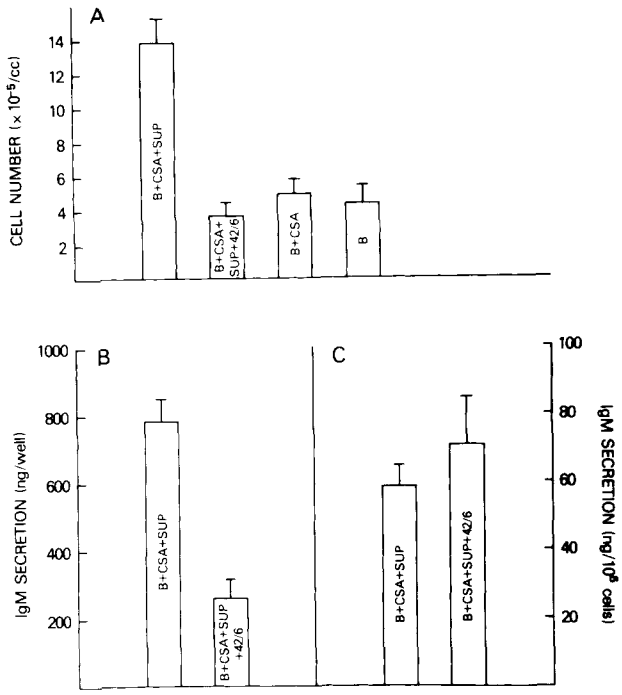

Fig. 4. Purified B-cell populations (T cell-depleted and monocyte-depleted) were cultured as described above. In A) cell number was determined on day 5 after stimulation. In B) IgM levels in culture supernatants were measured on day 5 and expressed as ng/well. In C) IgM secretion is normalized for cell number.

The effect of transferrin receptor blockade on the ability of mitogen to induce an elevation in IgM mRNA was studied and the results are depicted in Figure 6. When 42/6 is added with mitogen at culture initiation, no effect on IgM mRNA induction at 24 hr can be seen. Thus, even low numbers of transferrin receptors do not appear to be required for mitogen to induce a two- to threefold elevation in IgM mRNA within 1 day of culture initiation.

In order to determine if the elevated mRNA levels observed in the presence of transferrin receptor blockade could be functionally translated, we stained B cells for cytoplasmic IgM 3 days after culture initiation in the presence of mitogen, PHA supernatant, and 42/6. As can be seen in Figure 7, blockade of the transferrin receptor with 42/6 does not interfere with the appearance of cytoplasmic immunoglobulin in mitogen-stimulated B cells.

DISCUSSION

The present study demonstrates that the appearance of transferrin receptors on B cells is dependent on prior exposure of the cells to both CSA and PHA supernatant. Since B cells will not respond to supernatant alone or to CSA alone, our data are consistent with the hypothesis that exposure of B cells to CSA (or presumably antigen) results in the expression of a surface receptor for BCGF, analogous to lectin-induced stimulation of T-cell interleukin-2 receptors [5,12,25]. Exposure to BCGF-containing

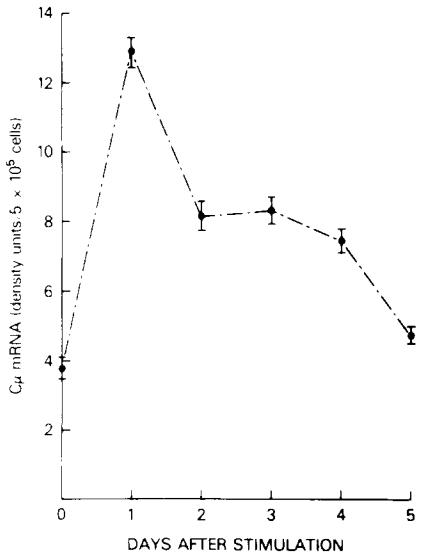

Fig. 5. IgM mRNA levels increase rapidly following mitogen stimulation of resting B cells. Each point is the mean of triplicate determinations. Values were obtained by scanning densitometry as described in the text.

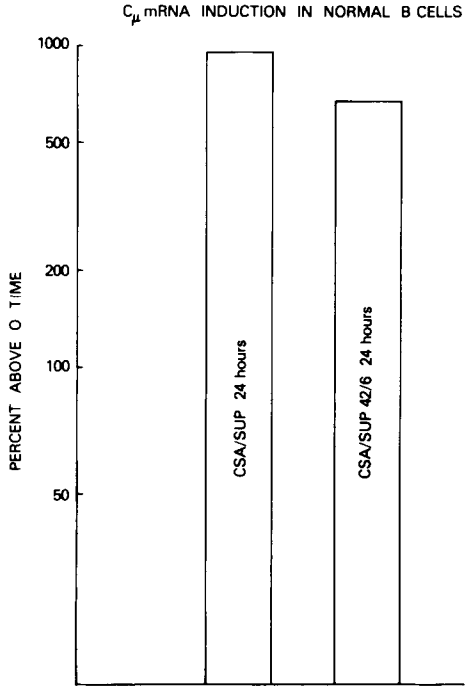

Fig. 6. Blockade of the transferrin receptor does not prevent the elevation in IgM mRNA levels induced by mitogen. 42/6 (5 μg/ml) was added at culture initiation and IgM mRNA was determined at 24 hr. The data are expressed on a log scale as percent above the level of IgM mRNA contained in resting B cells.

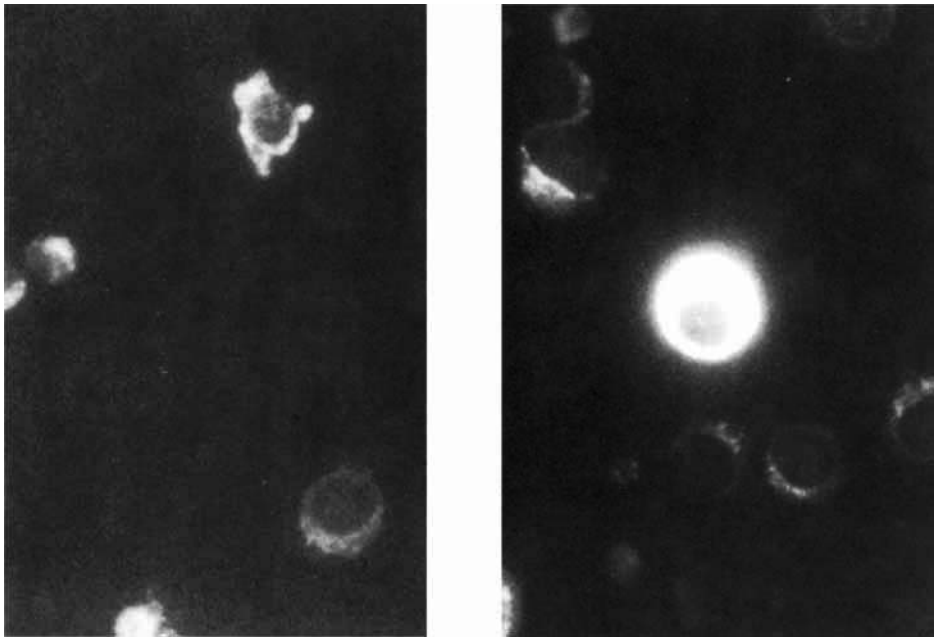

Fig. 7. Blockade of the transferrin receptor has no effect on IgM mRNA translation. Cells were stained for cytoplasmic IgM on day 3 following culture initiation with mitogen and PHA supernatant, with or without 42/6 (5 μg/ml). The left panel represents cells untreated with 42/6; the right panel shows cells treated with the antitransferrin receptor antibody. In both cases, intracellular IgM is clearly visible.

PHA supernatants then results in expression of B-cell transferrin receptors (just as exposure of IL-2 receptors-bearing T cells to IL-2 results in transferrin receptor expression on these cells). Once transferrin receptors have been expressed, thymidine incorporation and cell proliferation can commence. We have not only demonstrated that the cell's requirements for triggering thymidine incorporation and for expressing transferrin receptors are similar and that these two events follow similar time courses, but we have also shown that the actual modal number of transferrin receptors on a cell population is very closely correlated to the amount of thymidine that a cell population incorporates. Furthermore, an antibody to the transferrin binding site of the transferrin receptor (42/6) completely prevented the ability of CSA and PHA supernatant (containing BCGF) to induce B-cell proliferation, whether measured by thymidine incorporation or actual cell number. This ability of an antitransferrin receptor antibody to block B-cell proliferation in the presence of BCGF is consistent with similar results obtained with activated or IL-2-dependent T cells [5]. These findings support a model of lymphocyte activation in which proliferation is dependent on induction of the transferrin receptor in late G_1 phase of the cell cycle. Transferrin receptor induction is regulated by cell-specific growth factors (ie, IL-2, BCGF) whose own synthesis is tightly controlled. This model, which can apply to other tissues as well, implicates transferrin, a ubiquitous serum glycoprotein, as a major growth regulator of many cell types. Cells regulate their sensitivity to transferrin by regulat-

ing the level of transferrin receptors on their surfaces. Malignant transformation of lymphoid cells may result in (or be the result of) unregulated expression of the transferrin receptor; ie, constitutive expression of the transferrin receptor in the absence of homeostatic regulation by the cell-specific growth factor. In fact, most T-cell lymphomas do not possess IL-2 receptors nor do they respond to IL-2, yet they still require transferrin receptors for growth. The same may hold true for B-cell lymphomas, although BCGF and its receptor have not yet been well characterized.

We found that, although blockade of the transferrin receptor inhibited B-cell proliferation, B-cell blastogenesis was not prevented, suggesting that B-cell differentiation might be progressing normally in 42/6-treated cells. This observation allowed us to observe differentiation in B cells whose proliferation had been blocked at a known point, late G_1 phase of the cell cycle, without the use of potentially toxic substances such as hydroxyurea. By blocking the transferrin receptor we can specifically interdict proliferative signals and observe the ability of the cells to differentiate by determining their ability to synthesize and secrete immunoglobulin. In determining the requirements for induction of immunoglobulin secretion in this system, we observed that exposure to either CSA and PHA supernatant alone was essential but not sufficient to induce secretion. When CSA and PHA supernatant were added together, IgM secretion was detected by day 3 and increased markedly by day 5 of culture. When IgM secretion was expressed on a per cell basis, antitransferrin receptor antibody was in no way inhibitory. Although expressing secretion as a function of the number of cells at the end of the experiment may bias the data, a similar but opposite bias must be acknowledged in expressing secretion as a volume function wherein the cell number of different groups may vary by as much as 300% (due to antitransferrin receptor blockade of growth). These results show that B-cell proliferation may not be required in order for B cells to secrete immunoglobulin. In fact, when resting B cells were exposed to CSA and PHA supernatant, mRNA for the constant region of the IgM heavy chain can be shown to increase maximally above control levels within 18–24 hr after the beginning of the experiment, at least 36 hrs before any thymidine incorporation, transferrin receptor induction, or change in cell number can be detected. These results are similar in time sequence to those previously reported for the phorbol ester-induced immunoglobulin mRNA induction in chronic lymphocytic leukemia cells [22]. In this case, phorbol ester treatment drives these cells to secrete immunoglobulin in the absence of cell division [22, and Neckers et al, unpublished observations]. Furthermore, the mRNA induction we have observed in normal B cells, together with its concomitant translation, is not affected by 42/6 treatment.

Exposure of B cells to CSA (or antigen) results in expression of multiple surface receptors. Some of these receptors, such as that for BCGF, are involved in proliferation, whereas others, perhaps for T-cell replacing factor or B-cell diffentiation factor, are involved in differentiation [12,25–27]. Our data indicate that BCGF interaction with its receptor results in transferrin receptor expression and cell proliferation, whereas the interaction of differentiation factors with their receptors triggers production and secretion of immunoglobulin. Supernatants from mixed lymphocyte or mitogen-stimulated lymphocyte cultures obviously contain many factors, including the ones described above. Thus, using such supernatant preparations, the two processes of proliferation and differentiation cannot normally be distinguished. Because transferrin receptor expression is required for proliferation, but not differentiation,

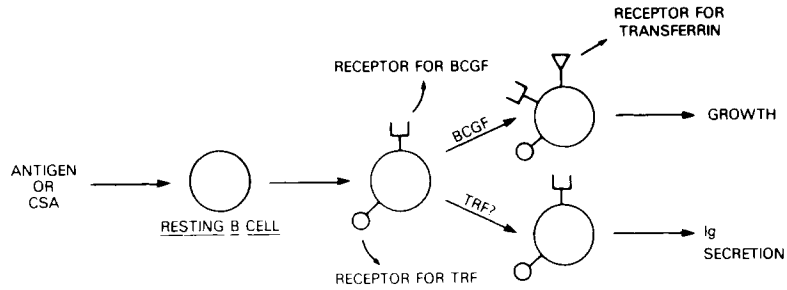

Fig. 8. CSA (or antigen) binding to resting B cells triggers the occurrence of multiple events as evidenced by appearance of receptors on the B cell for the T cell-derived factors BCGF and TRF (or other "differentiation factors"). Interaction of BCGF with B cells results in transferrin receptor expression and cell proliferation, whereas interaction of TRF with its receptor results in eventual immunoglobulin secretion.

use of antitransferrin receptor antibodies has allowed us to dissect the sequence of events involved in proliferation and immunoglobulin secretion. By doing this we have found that secretion can occur undiminished (when expressed on a per cell basis) in the absence of proliferation.

These results suggest a model for B-cell activation in which antigen exposure triggers the occurrence of multiple events—proliferation and differentiation of responding clones (Fig. 8). This concept is supported by studies using BCGF and TRF produced by T-cell hybridomas. Such a BCGF preparation will support proliferation but not secretion of immunoglobulin [9], whereas a TRF preparation will support immunoglobulin secretion but has no effect on proliferation [6].

In summary, we have demonstrated that B-cell transferrin receptors are induced by T cell-derived growth factors during the activation process and are required for proliferation. Using antibodies to transferrin receptors, we have shown that B-cell differentiation is apparently not dependent on transferrin receptors but in fact can occur independently of and concurrently with proliferation.

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