INDUCTION OF THE c-myc PROTOONCOGENE AFTER ANTIGEN BINDING TO HAPTEN-SPECIFIC B CELLS

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A central question in B cell biology concerns the potential role of the surface immunoglobulin (sIg) receptor for antigen as a means for signal transduction during the process of antigen-driven B cell activation. The original one-signal hypothesis (1) predicted that sIg functioned merely to focus antigen onto the surface of antigen-responsive cells. The validity of such a conclusion has been questioned by results from many studies (2, 3) in which anti-Ig reagents were used to successfully activate resting B cells. Most importantly, the crosslinking of sIg by such reagents has been shown to move resting (G₀) B cells into at least the G₁ stage of the cell cycle (4). However, when populations of hapten-specific B cells were stimulated with thymus-dependent (TD) antigens, the antigen-binding cells did not leave G₀ (5, 6), even though they were shown to bind and cap antigen (6). Recently, we have readdressed (7) this issue by searching for biochemical consequences of antigen binding to the sIg receptor. In this regard, we have shown that TD antigenic stimulation of hapten-specific B cells generated a detectable phosphatidylinositol cycle. In the present communication, we have shown that such antigenic stimulation of hapten-specific B cells induced elevated levels of c-myc mRNA. These observations lend further support to the concept that antigen binding to the sIg receptor results in the transduction of signals to the cell and directly implicates the active participation of sIg during the process of antigen-mediated B cell activation.

Materials and Methods

Animals. DBA/2 female mice were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and were used at 8-10 wk of age.

Preparation of TNP-ABC. The method used for the isolation of TNP-antigen-binding B cells (TNP-ABC) has been described elsewhere (8). The resultant cell preparations were 80-90% rosette forming cells (RFC).

Culture Conditions. The TNP-ABC were cultured in flat-bottomed 24-well plates (Costar, Cambridge, MA) at 2×10^6 cells/well in 1.0 ml of RPMI 1640 with penicillin, streptomycin, glutamine, 10% FCS (Sterile Systems, Logan, UT) and 50 μ M 2-ME. Cultures were incubated in a humidified atmosphere of 83% N₂, 10% CO₂, 7% O₂ at 37 °C. After the 12–18 h preculture period, the appropriate reagents were added in 10 μ l of complete medium and the cells were cultured for an additional 2 h.

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Analysis of c-myc mRNA Level. Total cellular RNA was isolated as described by Glisin et al. (9). For the dot blot analysis, aliquots of the recovered RNA plus 10 μg of yeast tRNA were applied to a nitrocellulose filter using a dot blot Minifold (Schleicher and

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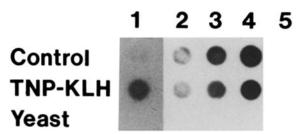


FIGURE 1. Dot blot analysis of c-myc mRNA levels in antigen-stimulated hapten-specific B cells. In lane 1, 1 µg of RNA isolated from control and TNP-KLH stimulated cultures and 10 µg of yeast tRNA were hybridized to a ³²P-labeled c-myc probe. Lanes 2–5 were hybridized to a murine ribosomal DNA fragment. Lane 2 was blotted with 25 ng of RNA, lane 3 with 50 ng of RNA, and lane 4 with 100 ng of RNA. 10 µg of yeast tRNA was blotted in lane 2. In lane 5, 100 ng of control and experimental RNA were treated with ribonuclease A for 20 min at room temperature before blotting. The TNP-ABC were 85% RFC.

Schuell, Inc., Keen, NH [10]). The hybridization reactions used 2×10^6 cpm/ml of the 32 P nick-translated probes (Sst I–Xho I fragment, derived from pSVc-myc-1 (11) and a murine ribosomal DNA fragment from λ qtWES MR100 [12] as previously described (10). Films were exposed for 24 h at -70° C using intensifying screens.

For Northern analysis, 4 μ g of total RNA from each experimental group and 1 μ g of poly(A)⁺ RNA isolated from MOPC 104E cells were fractionated by electrophoresis in 1% agarose gel. The gel was blotted to nitrocellulose and the hybridization reaction contained 2 × 10⁶ cpm/ml of the ³²P-labeled c-myc fragment. The filters were exposed for 18 h at -70° C using intensifying screens.

Results

To test for the ability of antigen binding to the sIg receptor to elevate levels of c-myc mRNA, populations of TNP-ABC were purified from DBA/2 mice (8). Such populations of cells have previously been shown to be resting B cells (>95% in G₀ by acridine orange analysis) (13) that are only activated to expansion and differentiation by TD antigens in the presence of carrier-specific Th cells (6, 14). TNP-ABC were divided into two groups, each consisting of two sets of cultures $(2 \times 10^6 \text{ cells/ml})$; Group 1 served as a control, while group 2 received 10 μ g/ ml of TNP-KLH (hapten carrier). After 2 h of stimulation, each group was harvested, the two sets of cultures in each group were pooled, and total cellular RNA was recovered for analysis of c-myc mRNA activity. Total cellular RNA from TD antigen stimulated or nonstimulated cultures was analyzed by dot blot hybridization to a ³²P labeled c-myc probe (Fig. 1). The TNP-ABC that were stimulated with hapten carrier displayed elevated levels of c-myc mRNA, in contrast to that seen with the nonstimulated cells (Fig. 1, lane 1). We saw similar results when the TNP-ABC were depleted of both macrophages and T cells. Also, the incubation of normal resting B cells with TNP-KLH did not result in the enhanced expression of c-myc mRNA (unpublished observation). As an internal control, serial two-fold dilutions of the cellular RNAs from both groups were hybridized to a murine ribosomal DNA probe (lanes 2-4). This control showed that equivalent amounts of RNA were analyzed in each experimental group. An additional control showed that the hybridization to the murine ribosomal probe was abolished by pretreatment of each RNA sample with ribonuclease (Fig. 1, lane 5).

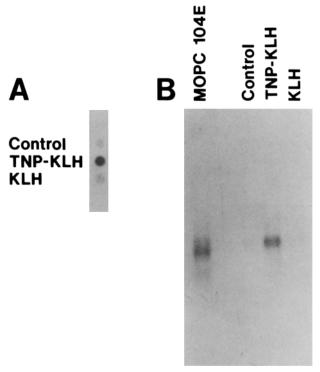


FIGURE 2. Dot blot and Northern analysis of c-myc mRNA levels in antigen-stimulated hapten-specific B cells. (A) 1 μ g of RNA isolated from the indicated groups was applied to the nitrocellulose filter. (B) For the Northern analysis, 4 μ g of total RNA from each experimental group and 1 μ g of poly(A)⁺ RNA isolated from MOPC 104E cells were fractionated by electrophoresis in 1% agarose gels. Both hybridization reactions used a ³²P-labeled c-myc probe. The TNP-ABC were 80% RFC.

Next, the TNP-ABC were divided into three groups, each group also consisting of two sets of cultures (2 \times 10⁶ cells/well). Group 1 served as a nonstimulated control, group 2 received 10 µg/ml of TNP-KLH (hapten carrier), and group 3 received 10 µg/ml of KLH (carrier). The total cellular RNA from each group was examined for the presence of c-myc mRNA by both dot blot analysis (Fig. 2 A) and Northern analysis (Fig. 2 B). In both types of hybridizations, RNA recovered from cells stimulated with hapten carrier displayed elevated levels of c-myc mRNA. In contrast, cellular RNA purified from carrier-stimulated cells showed the same low level of hybridization as the nonstimulated controls, indicating that carrier alone did not cause an increase in c-myc mRNA. This experiment showed that the hapten must be present for the elevation of c-myc mRNA, and predicted that binding by the sIg receptor of specific hapten mediated the observed effect. As an internal control, poly(A)+ RNA purified from MOPC 104E cells, which constitutively maintained high levels of c-myc mRNA, hybridized with our c-myc probe (Fig. 2B). As has been shown previously, the c-myc mRNA from MOPC 104E cells migrated faster than normal c-myc mRNA due to a recombination event within the MOPC 104E myc gene that has resulted in a shorter RNA transcript (15).

Discussion

Mitogenically stimulated quiescent lymphoid, fibroblast, and epithelial cells have all shown a marked increase in c-myc RNA levels (16, 17). Such results have suggested a potential role for the myc protooncogene during the process of cellular proliferation. Kelly et al. (16) showed that the increased levels of c-myc mRNA in both mitogen-stimulated B and T cells preceded the initiation of DNA synthesis. The stimulation of human B cells by antiimmunoglobulin reagents has been recently shown to likewise elevate levels of c-myc mRNA within 2 h of stimulation, with the levels of c-myc mRNA returning to normal by 24 h (18). Here we report for the first time that the specific antigenic stimulation of resting, hapten-specific B cells, in the absence of Th cell involvement, stimulated the enhanced expression of c-myc mRNA. These results confirmed the predictions made through the use of polyclonal stimulants by showing that such changes in c-myc mRNA levels occurred during a physiologic activation of B cells located in the G_0 stage of the cell cycle. Since the TD antigenic stimulation of such resting populations of B cells does not result in the progression of the cells into the G₁ stage of the cell cycle (5, 6), these findings strengthen observations reported in other systems that indicated that enhanced c-myc mRNA levels occurred while the activated cells were still located in G_0 (18–20) and may be causally related to the movement of such stimulated cells into the G₀* stage of the cell cycle (21).

We would like to propose the following sequence of events during the TD antigenic stimulation of resting B cells. The binding of antigen to the sIg receptor results in the transduction of a direct biochemical signal to the B cells. The initiation of the membrane-associated PI cycle (7) results in a variety of consequences to the cell, such as elevated cytosolic levels of Ca²⁺ (22), increased intracellular pH (23), and the phosphorylation of a number of membrane and cytoplasmic proteins (24). In addition, the activated PI cycle has recently been implicated in the increased levels of the myc mRNA (20, 25). In our view, the sum total of all these cellular changes, though insufficient by themselves to initiate the synthesis of ribosomal RNA and DNA, serve to prepare the cell for a major growth stimulus. In the case of the TD antigenic stimulation of B cells, this stimulus will be delivered through the direct interaction between Th cells and the antigen-binding B cells (6, 14, 26) and will result in the commitment of the B cell to DNA synthesis. Experimental observations from both the plateletderived growth factor stimulation of 3T3 fibroblasts (16, 17, 20) and anti-µ stimulation of human B cells (18) support this proposed model. These published observations indicated that stimulation of enhanced levels of c-myc mRNA does not commit the cell to DNA synthesis and that such a commitment requires delivery to the activated cells of an additional stimulus. Importantly, this additional stimulus, which in the case of the anti- μ -stimulated B cells consisted of T cell-derived soluble mediators, did not modulate c-myc mRNA levels (16-18).

In conclusion, we have shown that TD antigenic stimulation of hapten-specific B cells resulted in an elevation of the levels of c-myc mRNA. These results lend further credence to the direct participation of the sIg receptor for antigen during the process of TD antigenic stimulation of resting B cells.

Summary

The stimulation of hapten-specific B cell populations with the thymus-dependent antigen, TNP-KLH, was found to induce elevated levels of c-myc mRNA by 2 h. A similar treatment with carrier protein alone did not elevate c-myc mRNA above the level seen in the nonstimulated, resting B cells. These results indicate that antigen binding to the sIg receptor, in the absence of Th cell involvement, directly signals the antigen-binding cell and implicates the active participation of sIg during the process of antigen-mediated B cell activation.

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