Commentary

Connections between Signal Transduction Components and Cellular Responses Initiated by Antigen Receptor on B Lymphocytes

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Cells translate environmental cues into various cellular responses, including proliferation, differentiation, and death. In many cases, these responses are initiated through a specific interaction between an extracellular ligand and a membrane-bound receptor, which then triggers a series of biochemical events collectively termed signal transduction. Much progress has been made in the identification of second messengers and understanding the interactions between signaling proteins. Moreover, correlations between specific biochemical signaling events and a cellular response can be observed; however, demonstration of a direct cause and effect relationship is more difficult to achieve. Two observations have complicated this analysis. First, the binding of a ligand to its receptor can result in several different responses within the same cell, e.g., changes in growth characteristics, transcription patterns, adhesion properties, cytokine secretion profiles, et cetera. Any intracellular biochemical event induced by the ligand–receptor interaction might be involved in signaling one of these downstream responses and not the others, a subset of responses, or all of the responses. Second, cells of different differentiative stages can give different responses to the same ligand–receptor interaction, e.g., proliferation in one case and apoptosis in another.

To elucidate the different signaling pathways, it is important to understand which biochemical changes give rise to particular responses. Such insights may allow the rational development of new therapeutic agents that could alter particular branches of these signaling pathways. For example, under certain circumstances, it may be advantageous to use pharmaceutical agents that alter the adhesion properties of B lymphocytes without affecting their activation, proliferation, or differentiation responses. Several recent publications have described experiments that define connections between signaling components and specific downstream cellular responses initiated by engagement of the antigen receptor (BCR) on B lymphocytes.

B cells respond to cross-linking of their BCRs in several ways: (*a*) replication and terminal differentiation into plasma cells; (*b*) replication and differentiation to memory cells that then become arrested in the cell cycle; and (*c*) tolerance to self-antigens as a result of the induction of anergy and/or cell death (reviewed in references 1–3). Superimposed upon these responses are other phenotypic changes, which may include the expression of adhesion molecules to alter lymphocyte homing, the expression of particular cytokines to help influence the overall immune response, the switch from expression of membrane-bound Ig to secreted Ig, the switching of antibody isotype to elicit different effector functions, et cetera.

BCR-initiated signal transduction occurs through a multimeric protein complex centered around membrane immunoglobulin (reviewed in references 4–8). This complex includes the Igα and Igβ proteins (CD79a and b) that are noncovalently bound to the μ chain and are required for cytoplasmic signaling. When the BCR is cross-linked by antigen or anti-Ig antibodies, one of the earliest biochemical events (within seconds) is the activation of several tyrosine kinases that interact with the BCR complex, including four src family members Lyn, Fyn, Lck, and Blk, as well as another tyrosine kinase, Syk, which bears homology to the ZAP 70 protein associated with the TCR. Several targets for these tyrosine kinases have been identified, including GTPase-activating protein (GAP), phosphoinositide 3-kinase (PI3-K), the HS-1 protein, and phospholipase C-γ (PLC-γ) (9–11). As a result of phosphorylation, PLC-γ becomes activated to generate diacylglycerol (DAG) and inositol trisphosphate (InsP3). DAG then goes on to activate protein kinase C (PKC), whereas InsP3 generation stimulates the InsP3 receptor on the endoplasmic reticulum, resulting in the release of Ca^{++} from intracellular stores (12, 13).

Intracellular Ca^{++} responses after BCR engagement appear to have two components: a rapid, transient increase followed by a sustained oscillation of Ca^{++} concentrations within the cell. The Ca^{++} oscillation phase was first recognized when Ca^{++} measurements were made by examining kinetics in individual cells (14). When Ca^{++} measurements are made on a population basis, the combination of individual cell oscillations tend to cancel each other out. The initial transient response appears to be initiated by a release of Ca^{++} from intracellular stores, which in turn stimulates the influx of extracellular Ca^{++} (reviewed in references 15–17). Ca^{++} concentrations from this initial transient in-

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crease return to plateau levels within minutes. The sustained oscillations can last for hours and are associated with changes in the proliferative response (18). Using chimeric Igα and Igβ transfectants, activation through Igα can induce a transient response, whereas activation through Igβ gives a sustained oscillatory response (19).

Within the cytoplasm, various other signal transduction molecules have also been implicated in BCR signaling (reviewed in references 4–8), including components of the ras-raf-MAP kinase pathway. Within the nucleus, these biochemical changes result in altered transcription of important regulatory genes, including MHC class II and cellular protooncogenes such as c-*myc* and c-*fos*. The changes in protooncogene expression are likely to play an important role in regulating cellular proliferation and cell death.

An important goal in the field is to determine how these biochemical changes are integrated into particular cellular responses. For instance, does an increase in the concentration of intracellular Ca^{++} induce changes in cellular proliferation or upregulation of adhesion molecules, or is it required for both downstream responses? One approach to address these questions is to deplete the cells of a particular component that has been shown to be altered biochemically and then measure the cellular responses to BCR activation in these depleted cells. The prediction is that if a component is critical for only one response, then that response and not another would be abrogated in depleted cells.

This approach has defined the Lyn tyrosine kinase as a critical component for cell cycle arrest mediated by BCR cross-linking in lymphoma cells. Engagement of membrane-bound Ig has been found to induce two responses in certain B cell lymphomas: cell cycle arrest and apoptosis (20–23). If cells were treated with antisense oligonucleotides targeted to the *lyn* gene, however, the cell cycle arrest response was no longer observed, whereas the apoptosis response was maintained (22). Since treatment with the antisense reagent did not result in complete depletion of Lyn protein levels, a role for Lyn in apoptosis cannot be excluded. Indeed, B cells from *lyn* knockout mice may be resistant to anti-Ig–mediated apoptosis (Watanabe, T., personal communication). Nevertheless, these results indicate a critical role for Lyn in signaling cell cycle arrest, and they also suggest an early bifurcation in the signal transduction cascades originating from the BCR. Other studies using antisense oligonucleotides indicate that the Blk kinase is necessary for the apoptotic response (24).

The paper by Takata et al. (25) takes a similar approach to investigate the role of PLC-γ2 in downstream biochemical and cellular responses. They have exploited an elegant system, the DT40 chicken B cell line, in which the high incidence of homologous recombination after transfection (virtually 100% of integrants are targetted to the endogenous gene locus) greatly facilitates selection of gene knockout clones (26). They show that inactivation of the PLC-γ2 gene prevents the rapid increase in intracellular Ca^{++}, InsP3 generation, and the apoptotic response after BCR engagement. Not only does this indicate that PLC-γ2 is involved, but it also suggests that other PLC isoforms, which have been shown to be expressed in some B lymphocytes, are not able to substitute for the function of PLC-γ2 in DT40 for these biochemical and cellular responses. In contrast, no differences were observed in the protein phosphorylation pattern induced after BCR engagement in PLC-γ2–depleted cells, indicating that kinase activation is upstream of PLC-γ2.

Since the inactivation of PLC-γ2 abrogates both the calcium flux and the apoptosis response, does this mean that elevated intracellular free Ca^{++} is required to induce apoptosis? This is an important point since many signaling pathways include alteractions in intracellular Ca^{++} concentrations without the induction of apoptosis. An alternative explanation is that the requirement for PLC activation in the apoptosis response is the activation of PKC through the action of DAG rather than the induction of a calcium flux. One experiment to test this possibility would be to activate the PLC-γ2–depleted cells via the BCR in the presence of a calcium ionophore or together with activation of the muscarinic acetylcholine receptor (MuAR) with carbochol in appropriate transfectants. If the apoptotic response is restored, it could be concluded that Ca^{++} elevation is necessary for apoptosis. If apoptosis is still not observed, however, the result would suggest that another downstream effect of PLC-γ activation is required. Indeed, the fact that simultaneous activation of both the MuAR and BCR in PLC-γ2–depleted cells is still not sufficient to restore the apoptosis response (25) suggests that PLC-γ2 is playing an additional role in signaling apoptosis aside from the generation of DAG and InsP3. As a result of this experiment, the question of a requirement for increased Ca^{++} remains open.

The Ca flux may or may not be necessary for apoptosis induction, but it is clearly not sufficient. Ca^{++} fluxes induced by carbochol in MuAR transfectants in the PLC-γ2 knockout cells fail to induce apoptosis. Thus, some other effects of mIg-initiated signal transduction are also required to induce apoptosis. Obviously, apoptosis is a complex response. At some point, the signal for apoptosis must split into several branches to achieve all of the downstream effects. The question is, where does such branching occur in the signaling pathways?

Analysis of PLC-γ function has also been evaluated in DT40 cells in which either the *lyn* and *syk* genes have been inactivated by homologous recombination (27). In Syk-depleted cells, mIg cross-linking is no longer able to induce PLC-γ2 phosphorylation, phosphoinositide turnover, or changes in intracellular Ca^{++}. In contrast, in Lyn-depleted cells, all three of these mIg-induced responses are maintained, although the Ca^{++} flux is somewhat delayed. Activation of Syk is also partially reduced in Lyn-depleted cells (28), suggesting that whereas Syk alone may be capable of inducing the Ca^{++} response, Lyn may function to enhance the strength of the signal.

These findings support a model for negative growth sig-

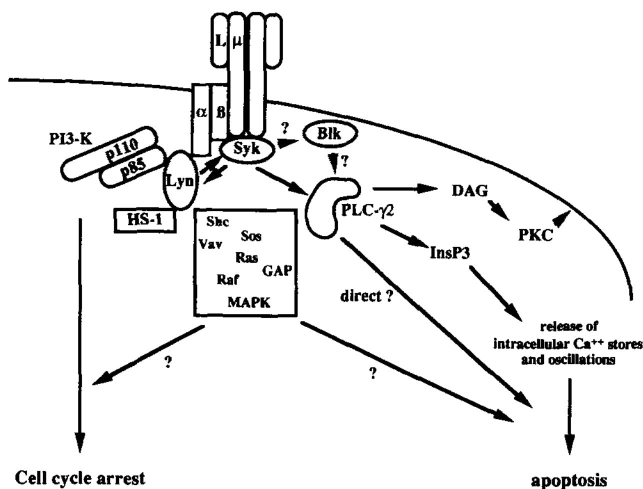

Figure 1. A model for the signaling pathways responsible for the induction of cell cycle arrest and apoptosis in B lymphocytes. See text for details.

naling originating from the BCR in which the branches for cell cycle arrest and apoptosis bifurcate at the level of the receptor-associated tyrosine kinases (Fig. 1). The Syk kinase appears to activate apoptosis through a signaling branch requiring PLC-γ and changes in intracellular free Ca^{++} concentrations. Apoptosis appears to be associated with a sustained Ca^{++} response involving the cycling of Ca^{++} in and out of intracellular stores; a rapid, transient increase shortly after antigen receptor engagement does not appear to be required. However, a sustained Ca^{++} response is probably not sufficient. It is possible that activation of the ras-raf-MAP kinase pathway is also involved, but this has not been tested directly.

The Lyn tyrosine kinase appears to be responsible for the induction of cell cycle arrest in a signaling branch that does not involve PLC-γ. Two elements downstream of Lyn in the cell cycle arrest pathway are likely to be HS-1 and PI3-K, since they have been shown to be major phosphorylation targets of activated Lyn. At this stage, the data do not address the involvement of Syk in cell cycle arrest, and it is possible that Syk is necessary for both responses.

An important implication of the analysis of these signaling pathways relates to therapeutic strategies. There are many studies underway to design new therapeutic agents aimed at interfering with signal transduction. This rationale is based on the observation that protooncogenes are frequently found to be components of signal transduction pathways, and that the genetic alterations associated with neoplastic transformation generally results in overexpression or constitutive activation. However, it is now clear from many studies, including those described above, that even malignant cancer cells can maintain growth regulatory pathways whose activation can arrest the cell cycle or induce suicide. Hence, agonistic molecules that can activate signaling pathways might also have therapeutic applications under appropriate circumstances (29). In this regard, antibodies aimed at receptors that activate these negative growth pathways are being evaluated for their antitumor effects in experimental animals and in humans.

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