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The Activation of Lymphocytes Is in Their *CARMA*

Lymphocyte activation via antigen receptors initiates adaptive immune responses. Two papers in this issue of *Immunity* demonstrate that CARMA-1, a CARD carrying member of the MAGUK family proteins, is essential for lymphocyte activation. CARMA-1 functions by coupling antigen receptor signals to NF-κB induction and JNK activation.

Lymphocyte activation via antigen receptors triggers many cellular responses, such as cytokine production, expression of cytokine receptors, cell survival, and entry into the cell cycle. Three transcription factor families implicated in these functions are NF-κB, NF-AT, and AP-1. These proteins are also induced in response to a variety of other stimuli, raising the possibility of signal-specific pathways for activation. The common feature of signals that induce NF-κB is activation of an IκB kinase (IKK). In the canonical pathway, IKKβ activation results in phosphorylation and degradation of IκBα, which releases IκB-associated Rel proteins to translocate to the nucleus. Signals initiated by antigens, TNFα or IL-1 induce NF-κB largely via this pathway. An alternate signaling cascade, initiated at receptors for BAFF and lymphotoxin β, activates IKKα and results in phosphorylation and processing of p100, the precursor to NF-κB p52. This releases RelB-containing complexes for gene expression.

The pathway from antigen receptors to IKK involves protein kinase C (PKC, θ in T cells and β in B cells) (Sun et al., 2000; Saijo et al., 2002; Su et al., 2002) and the caspase recruitment domain (CARD)-containing protein Bcl10 (Ruland et al., 2001). CARD is an approximately 110 amino acid region that mediates protein-protein interactions and has been implicated in many cellular processes including caspase recruitment (Bouchier-Hayes and Martin, 2002). Bcl10 contains an N-terminal CARD and a serine/threonine-rich C terminus that is phosphorylated in response to antigen receptor activation (Figure 1). The first evidence that another CARD-containing protein, CARMA-1 (CARD 11), played a special role in NF-κB activation came from a series of papers that described the functional consequences of CARMA-1 deficiency in T cell lines (Gaide et al., 2002; Wang et al., 2002; Pomerantz et al., 2002). CARMA-1 is a lymphocyte-specific member of the membrane associated guanylate kinase (MAGUK) family of proteins, and recruits Bcl10 to lipid rafts via CARD/CARD interactions after T cell receptor crosslinking. Two studies in this issue of *Immunity* now provide definitive evidence that CARMA-1 is essential for NF-κB activation via antigen receptors in both B and T lymphocytes. Furthermore, the requirement of CARD proteins is selective for NF-κB activation and not activation of other transcription factors such as AP-1.

Hara et al. (2003) explored the function of CARMA-1 by a conventional germline knockout approach. They find that the knockout mice have a profound defect in the development and/or survival of B-1 cells and NK cells, and a complete loss of B cell responses to LPS, anti-Ig antibody, and antigen, as well as a significant decrease in T cell responses to TCR ligation. Jun et al. (2003) identified mice with a point mutation in the coiled-coil region of CARMA-1 in a genome-wide chemical mutagenesis screen (see Figure 1). The mutation is presumed to disrupt the proper folding of CARMA-1, and thereby interferes with its function within the signaling complex. These mutant mice showed reduced numbers of B-1 cells, defective B cell responses to anti-Ig antibody and polysaccharide and protein antigens, and defective T cell responses to CD28-mediated costimulation. Intriguingly, Th1-dependent isotypes were selectively reduced and the reciprocal exaggeration of Th2 responses apparently resulted in a spontaneous allergic dermatitis. Both studies showed reduced activation of NF-κB and JNK in response to antigen receptor crosslinking in CARMA-1 mutant mice. Thus, two independent and very different approaches point to a critical function of CARMA-1 in B and T cell responses initiated by antigen receptors and costimulators.

The study of Jun et al. is an elegant demonstration of the potential of genome-wide chemical mutagenesis in a mammalian species. It is remarkable that increased surface IgM expression was the simple basis for identifying the CARMA-1 mutation and the prediction that B cells in these mutant mice would be defective in antigen receptor signaling. The thoughtful choice and application of ostensibly simple screening assays hold enormous promise for generating mutants that will continue to reveal critical pathways in lymphocyte development and activation.

Collectively, these studies lead to several important conclusions. First, lymphocytes have developed a unique signaling pathway that couples their antigen receptors to a transcription factor that is not lymphocyte specific. Essential components of this pathway are PKC, the CARD proteins, and IKK, but the connections between these components remain unclear. A plausible scenario is that PKC activation in response to antigen receptor ligation ensures Bcl10 recruitment and phosphorylation; this, in turn, activates IKK by a mechanism that is not yet defined. MALT1, a protein that interacts with Bcl10 (Lucas et al., 2001), may be an integral part of the signaling complex. The existence of such cell type-selective signaling pathways suggests that it may be possible to develop cell type-selective antagonists of NF-κB.

Second, CARMA-1$^{-/-}$ mice are deficient in JNK activation in response to antigen receptor signals. Thus, the CARMA-1 deficiency should not be viewed solely as a defect in NF-κB function. This is consistent with the stronger activation defects in these mice compared to single, or double, Rel knockouts that have been examined (Li and Verma, 2002). For example, it is possible that AP-1 *function*, but not DNA binding activity, may be affected in CARMA-1-deficient lymphocytes due to lack of JNK activity.

Third, the germline deletion of CARMA-1 appears to

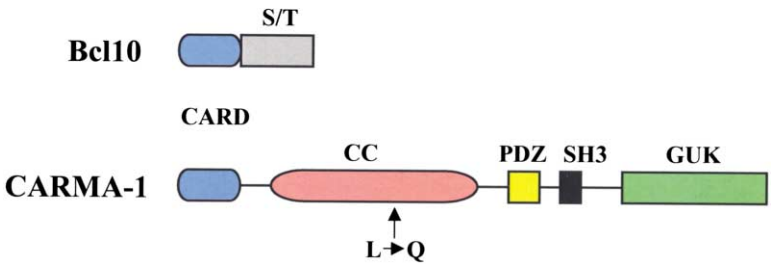

Figure 1. Schematic Representation of Caspase Recruitment Domain Proteins that Couple Antigen Receptor Signals to NF-κB Activation

CARD is shown in blue at the N termini. Bcl10 contains a C-terminal domain rich in serines and threonines (S/T). CARMA-1 contains, in addition to CARD, a coiled-coil domain (CC, pink), and PDZ (yellow), SH3 (black), and guanylate kinase (GUK, green) domains. The *un* mutation identified by Jun et al. is a single amino acid change within the CC domain of CARMA-1 as indicated.

cause more profound abnormalities than the point mutation. For instance, in the germline knockouts all B cell responses, including responses to LPS, are lost, serum Ig levels are greatly reduced, and T cells fail to respond even to anti-CD3 antibody. By contrast, in the mice with a CARMA-1 point mutation, LPS-induced B cell activation is normal (although anti-Ig-induced responses are defective), serum Ig levels are not reduced, and the greatest humoral immune defects are in Th1-dependent isotypes. Furthermore, T cells respond to anti-CD3, but the enhancement that is normally induced by CD28 costimulation is lost. One possibility is that the point mutation is "simply" a hypomorph that weakens the activity of the protein. In this case, it may be possible to develop quantitative relationships between NF-κB activation and various functional responses. For example, a stronger signal generated by LPS compared to anti-Ig in B cells may compensate for the handicapped mutant protein for NF-κB induction. Alternatively, the point mutation in the coiled-coil domain may disrupt certain protein-protein interactions, while retaining others. For example, a downstream effector of CD28 signals that may be recruited by the coiled-coil domain in normal circumstances could be selectively lost in the point mutation.

Finally, it is intriguing that the development or survival of only some cell types, such as B-1 cells and NK cells, are dependent on CARMA-1, even though the generation and survival of all lymphocytes, including B and T cells, require signals from antigen receptors. These findings suggest that B-1 cells and NK cells are more dependent on external stimuli for their generation and survival than are conventional B and T lymphocytes. It is unclear if this dependence reflects a requirement for antigen receptor signals or cytokines. Nevertheless, the results do suggest a fundamental difference in the developmental pathways of B-1 and NK cells, traditionally considered cells of innate immunity, compared with most B and T cells that are components of the adaptive immune system.

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Selected Reading

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Intracellular Pathogens and Antigen Presentation—New Challenges with *Legionella Pneumophila*

In this issue of *Immunity*, Neild and Roy (2003) examine the intracellular life of *Legionella pneumophila* in dendritic cells (DC) and macrophages, as well as the presentation of its antigens to CD4 T cells. *Legionella* is a particularly interesting bacterium because of the peculiarities inherent in its intracellular sojourn in phagocytes: it resides in an unusual vesicle characterized by ribosomes studded along its walls (Horwitz, 1983). In this compartment, *Legionella* proteins encoded by the *dot* gene inhibit phagosome-lysosome