In vitro functional analyses of T and B cells, DCs and macrophages.

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Method Article

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Abstract

Introduction

Immunoreceptor tyrosine-based activation motifs \(ITAMs) play crucial roles in antigen-receptor signaling in acquired immunity. Although receptors associated with the ITAM-bearing adaptors FcRγ and DAP12 on myeloid cells have been suggested to activate innate immune responses, the mechanism coupling those receptors to ‘downstream’ signaling events is unclear. The CARMA1–Bcl10–MALT1 complex is critical for NF-κB activation in lymphocytes but has an unclear role in myeloid cells. Here, we report that genetic deletion of the gene encoding the Bcl10-binding partner CARD9 resulted in impaired myeloid cell activation of NF-κB signaling by several ITAM-associated receptors. We also demonstrated that CARD9 is required for Toll-like receptor \(TLR)-induced dendritic cell \(DC) activation via MAP kinase activation. Whereas Bcl10−/− and Card9−/− mice exhibited similar signaling impairments for myeloid cells, Card11−/− myeloid cell responses were normal; and whereas Card11−/− lymphocytes are defective in antigen receptor-mediated activation, Card9−/− lymphocytes were not. Thus, the activation of lymphoid and myeloid cells through ITAM-associated receptors or TLRs is regulated by CARMA1–Bcl10 and CARD9–Bcl10, respectively.

Reagents

anti-CD3ε \(Pharmingen) anti-CD28 \(Pharmingen) PMA \(10 ng/ml) plus ionomycin \(1 μM), SEB \(5-500 ng/ml, SC BioScience) anti-IgM F\(ab’)2 fragment \(Jackson ImmunoResearch) anti-CD40\(BD Bioscience) rmIL-4 \(R&D Systems) LPS \(Sigma) CpG-DNA \(Hokkaido System Science) \[^{3}H]-thymidine \(Amersham) ELISA kit for mIFN-γ by \(R&D Systems) anti-CD16 \(BD Bioscience) anti-TREM-1 \(R&D Systems) anti-OSCAR \(provided from Dr. T.Takai) anti-MAIR-II \(provided from Dr. A. Shibuya) mouse gamma globulin \(Jackson ImmunoResearch) zymosan \(Sigma) oxidized-zymosan \(provided from Dr. N. Ohno) ELISA kit for IL-2, TNF, IL-6 and IL-12 \(BD Bioscience)

Equipment

Microbeta \(Perkin-Elmer)

Procedure

For proliferation assays 1. Stimulate T cells stimulated with anti-CD3ε \(Pharmingen), anti-CD28 \(Pharmingen), PMA \(10 ng/ml) plus ionomycin \(1 μM), and SEB \(5-500 ng/ml, SC BioScience) together with irradiated syngeneic spleen cells. Stimulate purified B cells with anti-IgM F\(ab’)2 fragment \(Jackson ImmunoResearch), anti-CD40, rmIL-4 \(R&D Systems), LPS and CpG-DNA). 2. After 2 or 3 days pulse the culture with 1 μCi of \[^{3}H]-thymidine \(Amersham) for 8 h and harvest the cells. Measure \[^{3}H]-thymidine incorporation with Microbeta \(Perkin-Elmer) or Matrix 96 \(Packard). **For cytokine production assays** Assay the culture supernatants of CD4+ and CD8+ T cells in triplicate for the

production of IFN-γ by ELISA \(R&D Systems). **For allogenic T cell response** Culture purified T cells \(5×10^4) from C57BL/6 mice for 48 h with irradiated \(30 Gy) spleen cells \(2.5×10^5) from BALB/c mice, and measure \[^{3}H]-thymidine incorporation in a similar manner. **For BMDCs or macrophage functional assay** 1. Stimulate BMDCs or macrophages with immobilized anti-CD16, anti-TREM-1, anti-OSCAR or anti-MAIR-II, or isotype-control rat IgGs in the presence of 10 µg/ml of soluble mouse gamma globulin as a FcR-blocker, or with Zymosan or oxidized-zymosan. 2. Collect the culture supernatant and analyze for IL-2, TNF, IL-6 and IL-12 production using respective ELISA kits \(BD Bioscience).

Timing

2 days