Expression of the CD40 Ligand in T Lymphocytes and Induction of IgE Isotype Switching

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Abstract

CD40 ligand (CD40L) delivers a **contact-dependent** signal to B cells which, in the presence of interleukin (IL)-4, drives immunoglobulin isotype switching to IgE. CD40L expression in T cells is transient, requires activation of protein kinase C and a rise in intracellular calcium concentration ($[Ca^{2+}]_i$), and is inhibited by cyclosporin A (CsA). CsA also inhibited **T-cell-dependent** IL-4-driven IgE synthesis. We have found that expression of CD40L is developmentally regulated. Expression of CD40L was restricted to mature **single-positive** thymocytes which, in the presence of IL-4, were capable of inducing B cells to undergo IgE isotype switching. CD40L expression was severely decreased in cord blood lymphocytes and was associated with a severely decreased ability to undergo **T-cell-dependent** IgE isotype switching.

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Immunoglobulin isotype switching to IgE in human B cells requires interleukin (IL)-4 and a contact-dependent T cell signal which is delivered by the CD40 ligand (CD40L) and which can be replaced by a CD40 monoclonal antibody (mAb) [1, 2]. IL-4 induces the transcription of a 1.8-kb ϵ germline mRNA which is not translated into a functional protein. Engagement of CD40 by its ligand or by mAb induces deletional switch recombination whereby the gene encoding the constant region of ϵ is juxtaposed to the rearranged variable region resulting in transcription of a mature 2.0-kb ϵ mRNA species.

We examine the expression of CD40L in T cells by cell surface staining and by Northern blot analysis [3]. CD40L was not detectable in resting T cells but was expressed transiently in activated T cells. CD40L mRNA and cell surface expression peaked at 3 and 6 h, respectively, and were undetectable 16 h after stimulation. Activation of protein kinase C (PKC) by phorbol myristate acetate (PMA) and increasing the intracellular calcium concentration by ionomycin were both required for CD40L expression. Next, we examined the effect of cyclosporin A (CsA), a naturally occurring

immunosuppressant, on the expression of CD40L. The complex of CsA and its endogenously expressed immunophilin, cyclophilin [4], inhibits the translocation of the cytoplasmic subunit of the transcription factor, nuclear factor of activated T cells to the nucleus [5] by binding to and inhibiting the activity of calcineurin [6], a Ca²⁺/calmodulin-dependent protein phosphatase. CD40L surface and mRNA expression were inhibited by pretreatment of T cells with CsA in a concentration-dependent manner with optimal inhibition at 100 ng/ml [3]. Inhibition of CD40L expression by CsA was associated with inhibition of CD40L-mediated T-cell-dependent B cell function. CsA inhibited T-cell-dependent IL-4-induced IgE synthesis in peripheral blood mononuclear cells, but did not inhibit T-cell-independent IgE synthesis induced by CD40 mAb and IL-4. These re-

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sults indicate that inhibition of IgE synthesis by CsA is exerted at the T cell level, and suggest that it is mediated by inhibition of CD40L expression.

We have also examined the developmental regulation of CD40L expression. CD40L was not detectable on resting or activated immature double positive (CD4⁺/CD8⁺) thymocytes. CD4⁺/CD8⁻ mature thymocytes expressed CD40L upon stimulation with PMA and ionomycin. Northern blot analysis revealed that CD40L mRNA was detectable in activated mature single-positive thymocytes. CD40L expression by mature thymocytes was associated with the capacity to drive immunoglobulin isotype switching in purified B cells. Because B cells could not be obtained from the thymus donors, we employed a mixed lymphocyte culture for induction of IgE synthesis [7]. In two different experiments, irradiated mature thymocytes were capable of driving IgE isotype switching in highly purified B cells in the presence of IL-4 and α -interferon- γ . These results demonstrate that the ability to express CD40L is acquired late in thymocyte development and that mature thymocytes are capable of providing B cells with the activation signals necessary for immunoglobulin isotype switching.

We also examined the capacity of newborn lymphocytes to express CD40L and to undergo IgE isotype switching [8]. PMA- and ionomycin-induced CD40L cell surface and mRNA expression were markedly decreased in newborn lymphocytes compared to normal adult lymphocytes. Newborn lymphocytes synthesized little or no detectable IgE in response to T-cell-dependent stimulation by IL-4 but synthesized IgE in response to T-cell-independent stimulation by CD40 mAb and IL-4. Therefore, decreased expression of CD40L in newborn lymphocytes was associated with a severely decreased ability to undergo T-cell-dependent immunoglobulin isotype switching.

In summary, CD40L, in the presence of IL-4, delivers a signal to B cells to undergo IgE isotype switching. Inhibition of CD40L expression by CsA is associated with the inhibition of T-cell-dependent IgE synthesis. The capacity to express CD40L is acquired late in thymocyte development and is associated with the ability to drive IgE isotype switching in B cells. In newborn lymphocytes, defective immunoglobulin isotype switching is associated with decreased expression of CD40L.

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44 Fuleihan/Ahern/Geha CD40L Expression and IgE Synthesis