Therapeutic Interventions Targeting CD40L (CD154) and CD40: The Opportunities and Challenges
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Abstract

CD40 was originally identified as a receptor on B-cells that delivers contact-dependent T helper signals to B-cells through interaction with CD40 ligand (CD40L, CD154). The pivotal role played by CD40-CD40L interaction is illustrated by the defects in B-lineage cell development and the altered structures of secondary lymphoid tissues in patients and engineered mice deficient in CD40 or CD40L. CD40 signaling also provides critical functions in stimulating antigen presentation, priming of helper and cytotoxic T-cells and a variety of inflammatory reactions. As such, dysregulations in the CD40-CD40L costimulation pathway are prominently featured in human diseases ranging from inflammatory conditions to systemic autoimmunity and tissue-specific autoimmune diseases. Moreover, studies in CD40-expressing cancers have provided convincing evidence that the CD40-CD40L pathway regulates survival of neoplastic cells as well as presentation of tumor-associated antigens to the immune system. Extensive research has been devoted to explore CD40 and CD40L as drug targets. A number of anti-CD40L and anti-CD40 antibodies with diverse biological effects are in clinical development for treatment of cancer and autoimmune diseases. This chapter reviews the role of CD40-CD40L costimulation in disease pathogenesis, the characteristics of therapeutic agents targeting this pathway and status of their clinical development.

Introduction

CD40 (TNFRSF5), a member of the tumor necrosis factor (TNF) receptor superfamily, is a signaling cell surface receptor. Sequence motifs involved in CD40-mediated signal transduction have been identified in the CD40 cytoplasmic tail that interact with the TNFR-associated factors (TRAFs) to trigger downstream signal cascades that in turn modulate the transcriptional activities of a variety of survival and growth-related genes.1-3 CD40 is expressed on B-cells at multiple stages of differentiation, monocytes, macrophages, platelets, follicular dendritic cells, dendritic cells (DCs), eosinophils and activated CD8+ T-cells.4-6 In non-hematopoietic tissues, CD40 is expressed on thymus and kidney epithelial cells, keratinocytes, synovial membrane and dermal fibroblasts and activated endothelium.7,9 The endogenous ligand for CD40 is CD40L (CD154, TNFSF5).4,5,7,10 Expression of CD40L on T-cells is tightly regulated; it is only transiently expressed on activated CD4+, CD8+ and γδ T-cells.11 Besides activated T-cells, CD40L is expressed by monocytes, activated B-cells, epithelial and vascular endothelial cells, smooth muscle cells and DCs. The functional relevance of CD40L expression on these cell types remains to be

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fully understood. However, expression of CD40L on activated platelets may participate in the pathogenesis of thrombotic diseases. The best characterized function of the CD40-CD40L interaction is in contact-dependent reciprocal interaction between antigen-presenting cells (APCs) and T-cells. On resting B-cells, binding of CD40L to CD40 promotes B-cell survival and activation, drives rapid expansion of antigen-activated B-cells and facilitates plasma cells and memory B-cell differentiation. CD40 signaling is required for germinal center formation, immunoglobulin (Ig) gene somatic hypermutation, affinity maturation and isotype switching. The physiological importance of CD40 signaling is illustrated by patients suffering from the X-linked hyper-IgM (XHIGM) syndrome. In this primary immunodeficiency, mutations in the CD40L locus abolish functional CD40-CD40L interaction. Disease manifestations include over-representation of circulating IgM and the inability to produce IgG, IgA and IgE. Consequently, patients have profoundly suppressed secondary humoral immune responses, increased susceptibility to recurrent infections and a higher frequency of developing cancer.

Genetic deletion of either the Cd40 or Cd40l locus in mice reproduces the major defects seen in XHIGM patients. The most prominent defect seen in the CD40−/− mice is the failure to form germinal centers. Thymus-independent IgG and IgM responses remain relatively normal in these mice, but antibody responses to thymus-dependent antigens and antibody class-switching are suppressed. Similar to the CD40−/− mice, the primary defects in CD40L−/− mice also reside in the B-cell compartment. In the T-cell compartment, functional differentiation of CD4 and CD8 cells have different requirements for CD40-CD40L costimulation compared to B-lineage cells. Helper Th-cell-mediated functions including local inflammatory reactions to lymphocytic choriomeningitis virus (LCMV) infection and ability to clear secondary viral infection are minimally affected in CD40−/− or CD40L−/− mice. Likewise, CD40L−/− mice can mount potent, primary virus-specific CD8 T-cell responses against LCMV, Pichinde virus and vesicular stomatitis virus. In contrast, the memory Cytolytic T-Lymphocyte (CTL) response in CD40L−/− mice is much less efficient than in wild-type mice even though memory CTL activity is detectable in CD40L−/− mice. Inadequate Th cell priming in these CD40L−/− mice is believed to be the main reason behind their diminished memory CTL responses. Functional T-cell-macrophage interaction in CD40L−/− mice is also hampered, resulting in altered macrophage-mediated inflammatory responses, heightened susceptibility to infection by Leishmania amazonensis and failure to generate a protective secondary immune response against the parasite.

CD40 signaling to DCs is probably the most important requirement in T-cell priming. CD40 ligation on DCs up-regulates MHC class II antigens, the costimulatory molecules CD80, CD86 and CD54, thereby promoting antigen presentation, inducing DC maturation and enhancing their costimulatory activity. Mature DCs stimulate activated T-cells to increase IL-2 production that facilitates Th and CTL expansion. CD40-stimulated DCs also secrete IL-12, TNFα, IL-8 and MIP-1α that favor Th1 cell differentiation and promote Th cell migration to sites of inflammation. In addition, CD40-activated DC cells presenting antigens in the context of MHC class I molecules are potent stimulators of CTL precursors, a process known as cross-priming, which is critical for cell-mediated immunity against viral infection and transformed cells expressing tumor-associated antigens. The importance of CD40 in CTL cross-priming is confirmed by the observation that administration of agonistic monoclonal antibodies (mAbs) against CD40 is sufficient to substitute the need of Th-cells for the generation of robust CTL responses.

In the reticuloendothelial system, CD40 ligation provides a pro-inflammatory signal. Triggering CD40 up-regulates the adhesion molecules CD54, E-selectin and VCAM-1 on monocytes, fibroblasts, keratinocytes, smooth muscle cells and activated endothelial cells. At the same time, the pro-inflammatory cytokines IL-1, IL-6, IL-12, IFNg and TNFα are secreted by these