

CD40/CD40 LIGAND INTERACTIONS IN IMMUNE RESPONSES AND PULMONARY IMMUNITY

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ABSTRACT

The CD40 ligand/CD40 pathway is widely recognized for its prominent role in immune regulation and homeostasis. CD40, a member of the tumor necrosis factor receptor family, is expressed by antigen-presenting cells, as well as non-immune cells and tumors. The engagement of the CD40 and CD40 ligands, which are transiently expressed on T cells and other non-immune cells under inflammatory conditions, regulates a wide spectrum of molecular and cellular processes, including the initiation and progression of cellular and humoral adaptive immunity. Based on recent research findings, the engagement of the CD40 with a deregulated amount of CD40 ligand has been implicated in a number of inflammatory diseases. We will discuss the involvement of the CD40 ligand/CD40 interaction in the pathophysiology of inflammatory diseases, including autoimmune diseases, atherothrombosis, cancer, and respiratory diseases.

Key Words: CD40, Immunity, B cells, Alveolar macrophages

INTRODUCTION

Various costimulatory molecules expressed as receptor and ligand pairs are involved in the fine tuning of the immune response by mediating both stimulatory as well as inhibitory signals. In the initiation of an adaptive immune response, in addition to a primary signal, which is provided by the engagement of the T-cell receptor with antigenic polypeptides, a class II major histocompatibility complex, subsequent secondary signals delivered by antigen-presenting cells (APCs) are required by T cells; these costimulatory signals promote T cell clonal expansion, cytokine secretion, and an effector function with other accessory signals.¹⁾ In general, costimulatory molecules are broadly divided into 2 main families: molecules from the CD28-B7 costimulatory family, such as cytotoxic T-lymphocyte antigen-4 (CTLA-4 or CD152) or programmed death (PD)-L1, and those from the tumor necrosis factor receptor (TNFR) superfamily such as OX40 or CD27.²⁾ Because of its essential role in immunity, one of the best characterized of the costimulatory pathways is the CD40 ligand (CD40L or CD154)/CD40 costimulatory pathway. The CD40L/CD40 pathway is not only required for effective T- and B-cell immune responses but also provides a critical initial step in the development of humoral and cellular immunity.

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The focus of this review is on the role of the CD40L/CD40 costimulatory system in the context of inflammation and clinical immunology, especially in the field of pulmonary medicine. Comprehensive reviews on the overall biology of the CD40L/CD40 system can be found in the literature.³⁻⁶⁾

CD40 and CD40L

CD40 is a 40- to 45-kD type I membrane protein and a member of the TNFR superfamily. The CD40 gene is located in chromosome 20 (q12-q13.2) and exists as a constitutional trimer complex on the cell surface. CD40 was initially characterized on B cells and is expressed on APCs, such as B cells, dendritic cells (DCs), macrophages, and monocytes, as well as on non-immune cells such as epithelial, endothelial, and mesenchymal (fibroblasts, myofibroblasts, synoviocytes, stellate cells, etc.) cells, platelets, and tumors.⁵⁾ This widespread expression coupled with the identification of two additional ligands for CD40, i.e., the 70 kDa mycobacterial heat shock protein⁷⁾ and the C4b binding protein,⁸⁾ indicates that CD40 may play a broader role in human physiology and disease pathogenesis. Since the CD40 molecule acts as a transmembrane signal transducer that leads to the activation of intracellular kinases and transcription factors within the cell, the engagement of CD40 regulates a wide spectrum of molecular and cellular processes, including the initiation and progression of cellular and humoral adaptive immunity. Since CD40 lacks intrinsic kinase activity in the cytoplasmic tail, its signals are transduced largely through the ligand-dependent recruitment of adaptor proteins of the TNF receptor-associated factor (TRAF) family. TRAFs not only couple CD40 to intracellular signalling components but also trigger the release of this signaling complex from CD40 to the cytosol by operating as E3 ubiquitin ligases to activate proximal protein kinases. That allows the activation of mitogen-activated protein kinases (MAPKs) pathways, including the extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK, the phosphatidylinositol 3-kinase (PI3K) cascade, the transcription factors nuclear factor- κ B (NF- κ B), and the signal transducer and activator of transcription (STAT).^{9,10)} These pathways act in concert to regulate many of the reported activities of CD40 in a cell-type and microenvironment-dependent manner.

CD40L is a 32- to 39-kDa type II transmembrane protein and a member of the TNF ligand superfamily (TNFSF, which consists of such as TNF, lymphotoxin, CD27L, CD30L, and FasL). The CD40L gene is located at chromosome X (q26.3-q27.1) and is expressed preferentially by activated CD4⁺ T cells as well as activated B cells and platelets, although it is also variably induced on monocytic cells, natural killer cells, mast cells, and basophils under inflammatory conditions.⁶⁾ A soluble form of CD40L (sCD40L) has been reported to express biological activities similar to the transmembrane form, triggering a CD40L/CD40 signalling cascade and also stabilizing the arterial thrombi by a β 3 integrin-dependent mechanism as an α IIb β 3 ligand.^{11,12)} Moreover, the integrins α 5 β 1 and Mac-1 were also recently identified as novel CD154 receptors expressed on many cell types.^{13,14)}

CD40L/CD40 interaction in immunity

CD40 interacts with CD40L, which is found primarily on activated T cells, playing a role in both humoral and cellular immune responses. After its ligation, CD40 is activated and then binds to TRAF proteins, which can propel a wide range of downstream signaling pathways with the subsequent production of surface and secreted molecules that ultimately impact on both humoral and cellular immunity, and eventually on inflammatory responses.⁵⁾

CD40L/CD40 interaction in humoral immunity

The critical role of a CD40L/CD40 interaction in the establishment of an effective thymus-

dependent (TD) humoral immune response became apparent and gained practical clinical relevance when patients suffering from the X-linked hyper-immunoglobulin (Ig) M syndrome (X-HIGM) were found to carry mutations in the CD40L gene.¹⁵ Such patients exhibit defective antibody production with a lack of circulating IgG, IgA, and IgE owing to their inability to switch the IgM isotype. Since patients with X-HIGM are thus immunocompromised in their humoral immune response, the activation of CD40 on B cells by CD40L is crucial for T cell-dependent B cell proliferation, differentiation, germinal center formation, as well as antibody isotype switching and affinity maturation, with those processes essential for the generation of memory B cells and long-lived plasma cells (Fig. 1). All of these *in vitro* events have been reproduced *in vivo* in mice carrying a genetic disruption of the CD40L/CD40 pathway.¹⁶⁻¹⁹ Mutations of the CD40 gene can also cause an autosomal recessive form of hyper IgM, which has been reported to be immunologically and clinically indistinguishable from the X-HIGM caused by CD40L deficiency.²⁰

We used CD40-deficient mice to investigate the role of humoral responses of antigen-specific IgG, IgE, and IgA in animal models of type I and type II allergic diseases.^{21,22} Since IgE does not explain all the symptoms encountered in the immediate hypersensitivity reaction to allergen, and since IgE-independent mechanisms have been speculated to induce the systemic type I hypersensitivity reaction, we investigated whether antigen-specific antibodies were indispensable to induce systemic anaphylaxis, and then whether the immunized antigen and this antigen-specific IgG induced both passive cutaneous anaphylaxis and passive systemic anaphylaxis. Furthermore, we confirmed the involvement of IgG in anaphylaxis by blocking with a monoclonal antibody (mAb) against the receptor for IgG (Fc γ R).²¹ In a murine model of Goodpasture syndrome, which is representative of type II hypersensitivity diseases, we reported the critical involvement

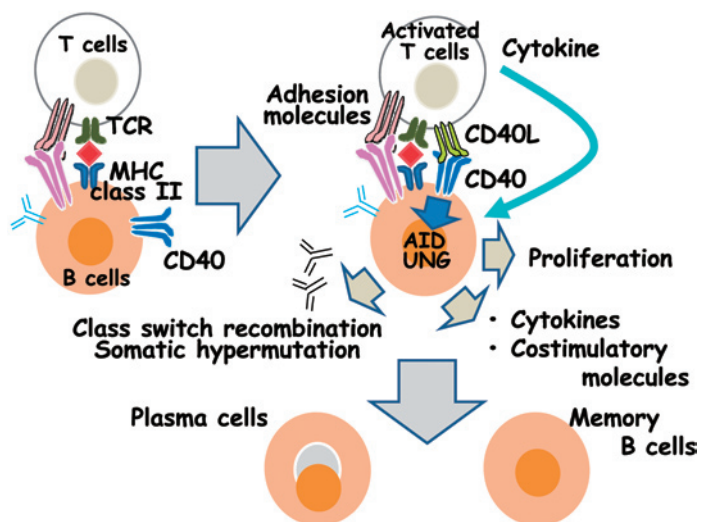


Fig. 1 B cell activation during cognate interactions with T cells and antigen-driven B cell differentiation. Engagement of CD40 on antigen-driven B cells undergoes clonal expansion, and their immunoglobulin genes undergo two unique alterations: class switch recombination and affinity maturation through somatic hypermutation and selection. These cellular and molecular processes promote antibody production (plasma cells) and the development of high-affinity B cell memory in response to foreign antigen exposure.

TCR, T-cell receptor; MHC, major histocompatibility complex; AID, activation-induced cytidine deaminase; UNG, uracil-DNA glycosylase.

of Fc γ R rather than the complement system in the development of disease using CD40-deficient mice to demonstrate the importance of antigen-specific IgG, IgE, and IgA.

Although CD40L/CD40 interaction was thought to comprise only a pair of CD40 and CD40L, the other receptors and ligands have also been recently identified. However, the roles and possible mechanisms of these newly found molecules in humoral immunity have not actually been reported in the available literature.

CD40L/CD40 interaction in cellular immunity

While initially thought to be involved only in regulating humoral immunity,³⁾ with the improvement in our understanding of the essential role of CD40L/CD40 in the development of TD humoral immunity, evidence began to accumulate indicating that CD40L/CD40 is also critical in the development of cellular immunity. Among a multitude of cellular functions, CD40L regulates the costimulatory activity of APCs, inducing B cells to upregulate the expression of CD80 (B7.1) and CD86 (B7.2) and DCs to upregulate the expression of CD54 and CD86.²³⁾ CD40L/CD40 interactions between CD4⁺ T cells and professional APCs influence T-cell priming and T-cell-mediated effector functions, while CD40L/CD40 interactions condition the APCs so that they are equipped for activation of CD8⁺ T cells. Additionally, ligation of CD40 on DCs leads to the production of various cytokines, including interleukin (IL)-8, TNF- α , and macrophage inflammatory protein (MIP)-1 α . Interestingly, as we have reported that CD40 stimulation plays an important role in two critical steps of cellular immunity, i.e., the generation of Th1 response and the activation of macrophages for the control of *Leishmania major* infection,²⁴⁾ CD40L/CD40 interactions have also proved important in the activation of macrophages and the amplification of the innate immune response to intracellular and extracellular pathogens. In addition, CD40L is one of the most potent stimulators of DCs that engulf debris and dying cells in tissues and induce DCs to produce IL-12, a cytokine that plays a key role in the polarization of Th1 immune responses.²⁵⁾

As might be expected, functional interactions between CD40 and CD40L are bidirectional, as exemplified by the observation that CD40-expressing APCs contribute to T cell activation. It has been demonstrated that CD4⁺ helper T cells proliferate poorly in response to antigen exposure, produce little IL-4 or interferon (IFN)- γ , and fail to generate antigen-specific T cell responses without a CD40L/CD40 pathway.²⁶⁾ That is most evident in the role of this CD40L/CD40 pair in the development of cytotoxic T cells to tumors, viruses, and alloantigens.^{27,28)}

As mentioned above, in addition to being expressed by normal classical lymphoid cells, both CD40 and CD40L are found not only on non-lymphoid hematopoietic cells, such as eosinophils, basophils, and monocytes, but also on non-hematopoietic compartments, including epithelial and endothelial cells, fibroblasts, myofibroblasts, synoviocytes, and stellate cells. As an effective way to communicate with numerous types of these cells and immune cells, a CD40L/CD40 interaction can result in the amplification of immune and inflammatory responses by the production of proinflammatory cytokines, chemokines, matrix metalloproteinases, prostaglandins, and the upregulation of adhesion molecules in these cells (Fig. 2). Moreover, without direct cell-cell interaction, sCD40L, a truncated soluble form of CD40L, retains its biological activity and acts as a cytokine. The main source of sCD40L is platelets, which produce nearly 95% of the plasma sCD40L pool. The role of circulating sCD40L in atherothrombosis is widely accepted.

Many studies have indicated that the CD40L/CD40 system participates in the pathogenic processing of chronic inflammatory diseases, such as diabetes, graft rejection, atherosclerosis, and cancer. An expression of CD40 is also evident in the majority of hematopoietic and epithelial malignancies where it has been implicated in oncogenic events.⁵⁾

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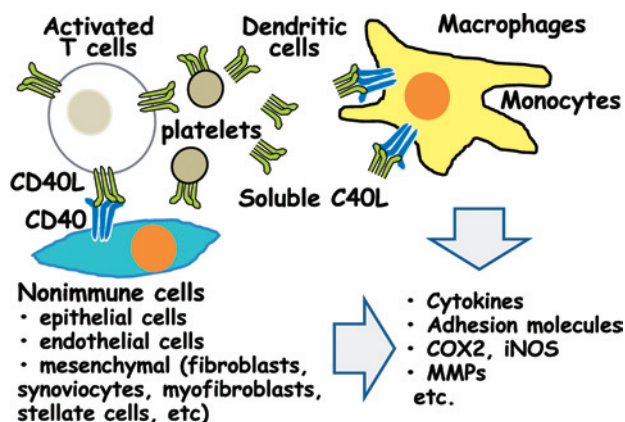


Fig. 2 Pleiotropic effects resulting from the interaction between CD40L (on T cells and platelets, or in soluble form) and CD40 on immune or non-immune cells.

When activated through the CD40 pathway, dendritic cells, macrophages, and monocytes induce a rather wide range of effects, which include the production of cytokines, cyclooxygenases (COX), an inducible nitric oxide synthase (iNOS), matrix metalloproteinases (MMPs), and an upregulation of cell adhesion molecules.

Activation of non-immune cells through the CD40 pathway induces a spectrum of effects similar to those observed in monocytic cells.

CD40L/CD40 interaction in disease pathogenesis

The chronic engagement of CD40 with a deregulated amount of CD154 has been implicated in a number of human pathologies, including atherosclerosis, autoimmunity and many inflammatory diseases.

Autoimmune disease

In a number of animal models, the CD40L/CD40 interaction has been shown to be involved in the onset of inflammatory disease, including experimental autoimmune encephalomyelitis, collagen-induced arthritis, thyroiditis, uveitis, inflammatory bowel disease, and diabetes.²⁹⁻³⁵⁾

Originally, the blockade of the CD40L/CD40 pathway disrupts the interaction between APCs and T cells, resulting in the lack of a secondary signal and the anergy of autoreactive T cells, which are a substantive contributor to the pathogenesis of autoimmune disease. Since CD40 has been demonstrated to be expressed on activated T cells and act as costimulatory molecules, the CD40L/CD40 pathway is now thought to be important for pathogenesis and development of autoimmune diseases, influencing not only the cognitive activation of APC but also that of T cells.³⁶⁾ Wagner et al. have reported that CD40 is also expressed on thymocytes and up to 50% of peripheral T cells in autoimmune prone strains of mice.³⁷⁾ The Wagner group has also demonstrated that CD40⁺ CD4 T cells are auto-aggressive and comprise a unique pathogenic T cell population in nonobese diabetic mice.^{38,39)}

There is now mounting evidence that CD40 is also expressed on activated T cells and may play a functional role in response to pathogens and the development and maintenance of autoimmune disease.

Atherothrombosis

CD40L/CD40 expression is known to be up-regulated in atheroma-associated cells. CD40L/CD40 interactions activate these cells by promoting the expression of molecules thought to

be involved in atherosclerotic plaque formation, such as adhesion molecules, cytokines, matrix metalloproteinases, and tissue factor. Furthermore, CD40L/CD40 ligation may promote leukocyte recruitment, participate in plaque weakening and play a prominent role in thrombotic events after plaque rupture. CD40L/CD40 (and possibly sCD40L) play a key role in atherothrombosis by acting as a link between platelets, inflammation, thrombosis, and atherogenesis.⁴⁰⁾

Cancer

Expression of CD40 is not restricted to normal cells but is also evident in many tumor cells, including nearly all B-cell malignancies and up to 70% of solid tumors. In fact, CD40 was first discovered as an antigen expressed in bladder carcinoma simultaneously with its identification as a receptor on B cells.⁴¹⁾ CD40 has been shown to provide both tumor-promoting and growth-inhibitory effects on CD40-positive cancer, depending upon the circumstances. As in normal B cells, CD40 ligation in certain B-cell malignancies causes an increase in the expression of many factors that protect the cell from apoptosis induced by apoptotic agents.⁴²⁾ Furthermore, the co-expression of CD40 and its ligand in malignant cells confers oncogenic properties, increasing their proliferation, motility and invasion,⁴³⁾ and their co-expression enables tumors to manipulate both T-cell and APC compartments, most likely contributing to the establishment of an immunosuppressive tumor microenvironment. On the other hand, CD40 induces apoptotic and antiproliferative activity on selected neoplastic cells.^{44,45)} Although the CD40 molecule lacks death domains, apoptosis may occur via the induction of membrane-bound cytotoxic ligands of the TNF family such as TNF- α , FasL and TNF related apoptosis-inducing ligand (TRAIL).⁴⁴⁾ Thus, CD40 ligation may directly mediate cytotoxic effects on neoplastic cells and/or indirectly by affecting tumor growth through the activation of host antigen-presenting cells, which then propel T-cell responses directed against tumors. Synergy develops if tumor antigens that are shed after a direct cytotoxic attack can be taken up by antigen-presenting cells during the activation process and confer tumor specificity on the resulting T-cell response. We investigated the anti-tumor activity of alveolar macrophages (AM ϕ) through a CD40-CD40L interaction. We established CD40L-expressing lung cancer cells by transfection with the CD40L gene. The CD40L-transfected lung cancer cells stimulated CD40 on AM ϕ , which was induced in the production of nitric oxide, TNF- α , and IL-12, and in tumoricidal activity in the presence of IFN- γ , which increased the expression of CD40 on AM ϕ . Furthermore, cocultivation of spleen cells with CD40L-transfected cancer cells induced specific cytotoxic T cells for wild-type cancer cells.⁴⁶⁾ Based on these observations of *in vitro* experiments, we investigated *in vivo* experiments and demonstrated that CD40L-transfected lung cancer cells were rejected in wild-type mice, syngeneic to cancer cells, by the induction of tumor-specific cytotoxic T-cell immunity. Those results were confirmed using CD40-deficient mice that were unable to generate a protective anti-tumor immune response following the inoculation of CD40L-transfected cells.⁴⁷⁾ On the other hand, in animal models of cancer, CD40L therapy was proven to reduce tumor growth to inhibit the angiogenesis-based promotion of tumor growth. Accumulating evidence suggests that the CD40 pathway played its emerging role in the treatment of malignancy. Recently, preliminary findings emerging from clinical trials indicate that antibodies to CD40 can induce immune modulation and clinical responses in cancer patients.

Respiratory disease

Respiratory diseases range from the mild and self-limiting such as the common cold to the life-threatening such as bacterial pneumonia or pulmonary embolism. CD40-CD40L interactions have reportedly been involved in the pathophysiology of many inflammatory lung disorders, such as bronchial asthma, acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), and interstitial pneumonia. To investigate the contribution of CD40-CD40L interactions to bronchial

asthma, antigen-induced inflammatory responses in the airways have been examined, and their engagement increases the production of inflammatory mediators, suggesting that CD40 ligation favors airway inflammation.⁴⁸⁾ Recently, silencing the expression of CD40 using small interfering RNA in ovalbumin (OVA) -sensitized mice has also been discovered to attenuate allergy through the inhibition of DC and B cell functions and the generation of regulatory T cells. During allergen sensitization, cooperation between T and B cells through a CD40-CD40L interaction is an indispensable signal to trigger isotype class switching towards IgE. In a model of mice sensitized with grass pollen, the administration of a blocking anti-CD40L antibody during, but not after, the sensitization phase has been established prevents the production of allergen-specific antibody.⁴⁹⁾ In a mouse model of hyperoxic lung injury, an anti-CD40L antibody, either before or after oxygen exposure, was remarkably effective in reducing, and in many cases preventing, lung injury.⁵⁰⁾ In our group, we investigated the lipopolysaccharide (LPS)-induced lung injury model, focusing on the role of activated AM ϕ through CD40.⁵¹⁾ Activated AM ϕ are known to constitute a critical modulator of the lung inflammatory response through the production of various mediators. Without CD40, LPS-induced ALI was significantly reduced in its histological degree of injury and recruitment of neutrophils into the lung. Activation of AM ϕ through CD40 is involved not only in its amplification by the interaction with CD154 but also in the development of ALI by CD40 itself. Furthermore, we observed the accumulation of surfactant and *Pneumocystis* organisms in the lungs of CD40-deficient mice after long-term exposure to OVA to establish the model of bronchial asthma.⁵²⁾ Those mice are a model of secondary pulmonary alveolar proteinosis resulting from *Pneumocystis* infection. In CD40-deficient mice, although the amounts of surfactant-associated protein (SP) production were up-regulated, AMs showed neither phagocytic dysfunction or abnormal differentiation. The CD40-CD154 interaction plays an important role not only in the regulation of SP production, but also in switching the condition within the alveoli from innate immunity to acquired immunity. Since the lung is exposed to inhaled particles and pathogens, the actions of an innate host defense system including the pulmonary collectins such as SP-A and SP-D are important to clear them. There is a possibility that once acquired immunity is developed, activated T cells expressing CD154 could migrate to the alveoli and regulate the production of surfactant in immunocompetent individuals (Fig. 3).

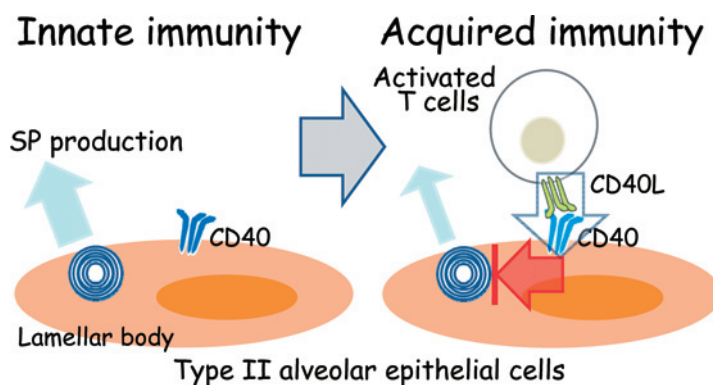


Fig. 3 Immunoregulation of surfactant-associated protein (SP) production in alveoli. Type II alveolar epithelial cells produce four SPs (SP-A, SP-B, SP-C, and SP-D). SP-A and SP-D, which belong to a collectin subgroup as pulmonary collectins, orchestrate innate immunity in the lung. After acquired immunity has supervened, type II alveolar epithelial cells markedly lower the production of SPs by the interaction of CD40 on themselves with CD154 on activated T cells.

CONCLUSION

Our studies on CD40 started to investigate its role in B cell differentiation. However, since evidence began to accumulate indicating that CD40L/CD40 was also critical in the development of cellular immunity, the CD40L/CD40 interaction ultimately had an impact on both humoral and cellular immunity, and eventually on inflammatory responses. The engagement of the CD40 with deregulated amounts of CD154 has been implicated in a number of inflammatory diseases. Although clinical trials using anti-CD154 mAb were halted following the evidence of unanticipated thromboembolic side effects, targeting CD40 rather than CD154 was thought to allow an interruption of the CD40/CD154 interaction between T cells and APCs. A blockade of the CD40-CD40L interaction should provide promising and novel therapeutic methods.

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