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Review

The CD40/CD154 receptor/ligand dyad

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Abstract. Until recently, the expression and primary function of the cell surface receptor CD40 and its ligand CD154 were considered restricted to B and T lymphocytes, and their interactions required for the thymus-dependent humoral response. However, current work from several groups challenges this view of the CD40/CD154 dyad as a mere mediator of lymphocyte communication. A variety of non-lymphocytic cell types express both receptor and ligand, including hematopoetic and non-hematopoetic cells, such as monocytes, basophils, eosinophils, dendritic cells, fibroblasts, smooth muscle, and endothelial cells. Accordingly, ligation of CD40 mediates a broad variety of immune and inflammatory responses, such as the expression of adhesion molecules, cytokines, matrix-degrading enzymes, prothrombotic activities, and apoptotic mediators. Consequently, CD40 signaling has been associated with pathogenic processes of chronic inflammatory diseases, such as autoimmune diseases, neurodegenerative disorders, graft-versus-host disease, cancer, and atherosclerosis. This review focuses on the synthesis and structure of CD40 and outlines CD154/CD40 signaling pathways, and emphasizes the previously unexpected importance of the CD40/CD154 receptor/ligand dyad in a spectrum of immunoregulatory processes and prevalent human diseases.

Key words. CD154; CD40; immunity; inflammation; arteriosclerosis.

1. Introduction to the CD40/CD154 dyad

Since its original discovery more than a decade ago, the CD40/CD40L receptor/ligand dyad has received increasing interest in the scientific community, as demonstrated by the steadily increasing number of publications (initiating in 1986 with 1 report, the number of Medline-listed publications increased to 594 in 2000, totaling 3026 reports on CD40, CD40L, CD154, Bp50, CDw40). CD40 was originally discovered in immunohistochemical studies employing an antibody detecting a 50-kDa protein (originally termed Bp50) on the surface of B lymphocytes [1–3]. Those early studies revealed that the expression level of this molecule varied with the status of B cell activation, fluctuating with progression through the cell cycle as well as with B lymphocyte differentiation and survival upon ligation of a then unknown ligand [4]. Later studies showed that this activation of B lymphocytes required direct contact with T helper cells rather than soluble lymphokines [5, 6]. Further work showed that the B lymphocyte-activating function required de novo protein synthesis, and followed stimulation by anti-CD3 antibodies, phorbol myristate acetate (PMA), or concanavalin A [7]. Moreover, early studies showed that the T helper cell induced B cell cycle entry into G1 did not result from an increase in expression of then recognized surface markers, such as CD3, CD4, LFA-1, intracellular adhesion molecule (ICAM)-1, class I major histocompatibility

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complex (MHC) molecules or Thy-1. Ultimately, the identity of the polycolonal, antigen-non-specific, and MHC-unrestricted B cell activation triggered by activated T helper cells via a non-polymorphic cell surface molecule was elucidated by cloning of the murine and human ligand for CD40, termed CD154 [previously also referred to as CD40 ligand (CD40L), gp39, TRAP, or TBAM], an integral membrane protein thought restricted to activated CD4+ helper T cells [8–12].

Further studies on the biological function of the receptor/ligand dyad established interactions of CD154 with its receptor CD40 on B lymphocytes as a crucial process in T cell-dependent B cell differentiation and activation [13]. Further evidence for this conclusion was provided by studies employing blocking antibodies which prevented an immune response to T cell-dependent antigens [13, 14] and affected the development of memory B lymphocytes and germinal centers [15]. In accordance with the proposed primary function of CD40/CD154 interaction, mutations in the ligand, interfering with receptor interactions, were identified as the cause of the X-linked immunodeficiency hyper IgM-syndrome (HIGM) [16], a disease associated with drastic or complete inhibition in the T cell-dependent humoral immune response.

Aside from the importance of CD154/CD40 interaction for appropriate immune responses, more recent studies have demonstrated a much broader expression pattern of both the ligand and its receptor, and associated CD154/CD40 interactions with diverse physiological and pathological processes: CD154, originally described on T helper cells, is also expressed on non-lymphoid cells, such as epithelial cells, monocytes, dendritic cells, fibroblasts, smooth muscle cells, and endothelial cells

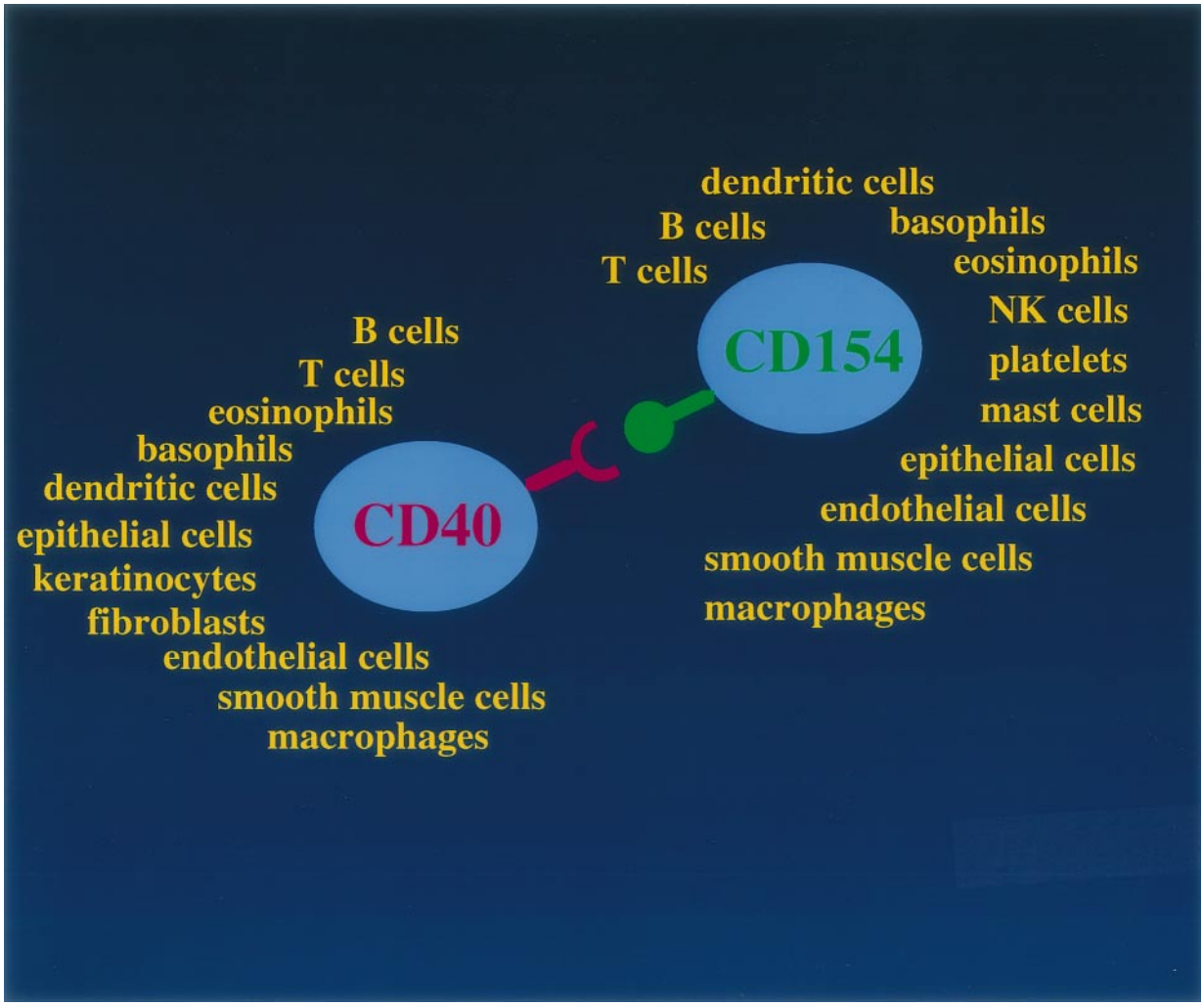

Figure 1. Human cell types expressing CD40 and CD154.

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[17–23]. Most of these ligand-bearing cells also express its counterpart CD40 [18–24]. The discovery of the broad distribution pattern of CD154 (see fig. 1) further implicated this receptor/ligand dyad in inflammatory and immune responses underlying various diseases, such as arthritis, cancer, atherosclerosis, lupus nephritis, and acute or chronic graft-versus-host disease.

To provide the reader with easy access to sections of interest, the review has been structured in divisions and subdivisions as outlined in table 1.

2. Structure of CD154

2.1. Gene

CD154, a type II transmembrance protein, belongs to the tumor necrosis factor (TNF) gene superfamily, consisting of molecules such as TNF, CD27 ligand, CD30 ligand, Fas ligand, lymphotoxin, and Ox40 ligand [25]. Human CD154 cDNA was obtained by screening activated peripheral blood T lymphocytes with the respective murine probe [8, 10, 11]. The 13-kb DNA sequence for CD154 shares 80% overall homology with its murine counterpart [26]. Mapped to chromosome X [10], region q26.3–q27.1, the gene is composed of five exons and four intervening introns [27], and encodes a 2.3-kb mRNA, which upon transcription yields a polypeptide consisting of 261 amino acids (260 amino acids for the murine ligand) (fig. 2). The large, 215 amino acid-long (214 amino acids for the murine ligand), cysteine-enriched (four cysteine residues) carboxy-terminal extracellular domain is mainly encoded by exons II–V, whereas the small transmembrane (24 amino acids) and amino-terminal intracellular (22 amino acids) domains are encoded by exon 1 [27, 28]. As characteristic for members of the TNF gene superfamily, the amino acid sequence of CD154 was suggested to align in the typical TNF-fold and bind to its receptor as a multimer [25, 29, 30], a hypothesis confirmed by recent studies, as described below.

2.2. Protein

Beside the cell-associated full-length 39-kDa protein, shorter soluble forms of the ligand have been described

with a molecular weight of 31, 18, and 14 kDa [31–33]. Recently, an additional 33-kDa CD154 species has been reported in murine B cells [33]. Further studies demonstrated that CD154 builds heteromultimeric complexes, consisting of full-length and/or smaller fragments, on the cell surface of human T lymphocytes [34]. The 18-kDa form, which lacks the cytoplasmic tail, the transmembrane region, and parts of the extracellular domain, is functional and is considered the soluble form of this 'cytokine' [32, 33]. However, the mechanism of solubilization remains unknown. Future studies will have to test whether the generation of soluble CD154 shows any analogy to the enzymatic cleavage pathway well characterized for other members of the TNF gene superfamily, such as FasL or TNF [35, 36].

2.3. Molecular structure

Despite the structural homology of the CD154 receptor-binding domain to other TNF gene superfamily members [37], considerable differences exist in several loops, including those predicted to be involved in CD40. In particular, neither the extracellular domain of CD154, which consists of a 75-amino acid spacer region immediately adjacent to the membrane-spanning region, nor the receptor-binding domain, consisting of two overlying β sheets as determined by the X-ray crystal structure, are shared with other TNF gene superfamily members [38]. The interaction between the receptor and its ligand is stabilized by charged residues, with CD154 presenting basic chains (K143, R203, R207) and CD40 presenting acidic side chains (D84, E114, E117) [39]. A wall of hydrophobic residues surrounds the polar interacting groups in the CD154/CD40 complex [39]. These studies extended earlier experiments that employed structure-based sequence alignments, side-directed mutagenesis, and receptor-ligand binding assays, demonstrating the importance of the CD154 residues K143, Y145, Y146, R203, and Q220, as well as the CD40 residues Y82, D84, E74, E117, and N86 for the binding process (probably by determining the structure of the protein, rather than by direct involvement in binding), indicating that CD154/CD40 interactions localize in two residue clusters [29, 30, 40]. Comparison of the CD154 and CD40 residues involved in the binding process with those residues identified in TNF-β/TNF receptor interactions demonstrated similarities but also unique features in the CD154/CD40 receptor ligand dyad. X-ray crystallographic and mutagenesis studies have suggested various models of human CD154 structure [37–41].

3. Structure of CD40

3.1. Gene

Bearing the type I extracellular binding motif and further structural homologies, CD40 is considered a member of

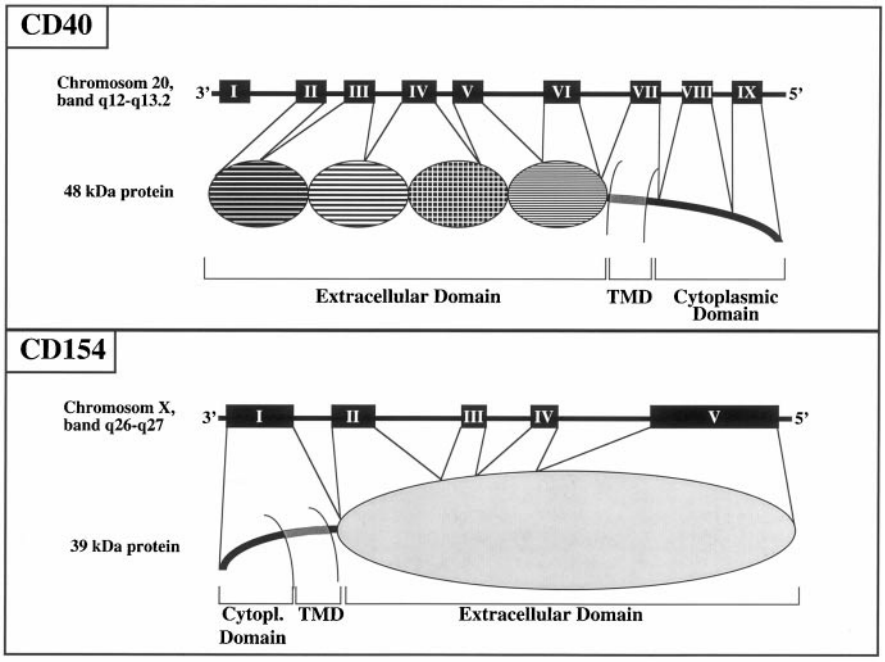

Figure 2. Organization of the human gene and protein for CD40 and CD154. Shown are the genomic (top) and protein (bottom) organization of CD40 (upper panel) or CD154 (lower panel). Exons are indicated as black boxes, labeled with the respective exon number.

the TNF receptor superfamily, encompassing the TNF receptor type I (p55-TNFR, CD120a), TNF receptor type II (p75-TNFR, CD120b), low-affinity nerve growth factor receptor, CD27, CD30, CD95 (Fas/Apo), Ox40, and 4-1BB [25]. Although the protein was identified on B lymphocytes by monoclonal antibodies in 1984 [1], another 5 years passed before the cDNA encoding CD40 was isolated from a mammalian expression library derived from Burkitt lymphoma Raji cell line revealing extensive homology with the nerve growth factor receptor [42]. The CD40 gene encodes a single 1.5-kb mRNA species and maps to human chromosome 20, bands q12–q13.2 [43, 44] and murine chromosome 2 [45]. Transcription of the gene results in a 277-amino acid membrane-bound protein that consists of a 22-amino acid signal sequence, a 171-amino acid extracellular domain, a single 22-amino acid transmembrane domain, and a 62-amino acid cytoplasmic domain (fig. 2). As typical for members of the TNF receptor superfamily, CD40 is characterized by a repetitive amino acid sequence pattern of four cysteine-enriched subdomains, typically consisting of six cysteines forming three disulfide domains. The intracellular domain of CD40, however, does not display a close relationship to other members of the family. The cytoplasmic domain of CD40 contains at least two major signaling determinants that include threonine 227 and 234, as discussed in detail below [46, 47]. Human and murine CD40 share 62% homology at the amino acid level throughout their open reading frames (78% for the intracellular domain, 100% for the C-terminal 32 residues). Activation of the murine gene results in two mRNA species (a. 1.4- and 1.7-kb form) by alternative usage of polyadenylation signals in the 3′ untranslated region, providing, however, identical coding sequences [48]. The 16.3-kb murine genomic DNA sequence for CD40 encodes nine exons [45].

3.2. Protein

Translation of the 1.5-kb CD40 mRNA generates an immunoreactive protein with a molecular weight of 43–50 kDa, mostly reported as a doublet consisting of a 43-kDa and a 47-kDa protein [4, 49]. Furthermore, dimer formation has been described in B lymphocytes [49]. Recent studies describing two CD40 epitopes that are differentially distributed on subpopulations of dendritic and epithelial cells indicate the possibility that this receptor might be expressed in a cell type-specific fashion [50].

3.3. Molecular structure

Relatively few experimental data exist regarding the molecular structure of CD40. Models of the receptor refer mostly to information obtained with the TNF receptor [51, 52], in which the 44-amino acid, six-cysteine residue, classical domain established the prototypical structure of this protein family. Indeed, several studies demonstrated that the domains involved in the interaction of CD40 with its ligand essentially correspond to those in the homologous TNF system, and a detailed three-dimensional model of the extracellular region of CD40 provided further evidence for structural homology with the TNF receptor [41]. Binding and mutagenesis analysis, X-ray crystallography, as well as molecular modeling experiments employing single and double amino acid-substituted proteins implicated the acidic side residues Y82, D84, E74, E117, and N86 in CD154 binding [29, 30], and further suggested that polar interactions in the surface of CD40, similar but inverted to those in the TNF receptor, stabilize the receptor-ligand complex [39].

4. Expression of CD154

4.1. Regulation of CD154 protein expression

Fusion proteins of the extracellular CD40 domain and the Fc region of human IgG1 have been employed to identify CD154 and to study the regulation of CD154 synthesis. Original studies demonstrated CD154 expression on activated mature but not resting human CD4+ T lymphocytes [8–12]. Within the T lymphocyte population CD154 is also found in Th0, Th1, Th2, CD8+, cells, as well as CD4−/CD8−δ T cell receptor (TCR)+ and $\alpha\beta$TCR+ T lymphocytes [53–56]. In accordance with this cellular distribution, immunhistochemical analysis demonstrated CD154+ T lymphocytes in the outer layers of germinal centers, the interfollicular T cell-rich areas, and the thymus [57, 58]. Although expression of CD154 is not restricted to T lymphocytes (as outlined below), our knowledge concerning the regulation of CD154 synthesis derives primarily from the original source. T lymphocytes can express CD154 on the cell surface as soon as 5 min after activation, indicating the ability to expose preformed CD154 [58]. The expression of the ligand on activated T lymphocytes, however, is transient, peaking 6 h after activation and declining over the following 12–24 h [54, 59]. Further studies established similar patterns of regulation on other CD154-expressing cell types, and revealed de novo synthesis of the protein following cell activation [18, 60]. The activation-induced expression of CD154 on T lymphocytes is regulated primarily by signaling through the TCR and might be enhanced by accessory molecules. However, these findings are controversial, since even in the absence of costimuli, CD154 expression in T lymphocytes is inducible by exposure to anti-CD3 or anti-CD2 antibodies [61, 62]. Accordingly

Roy et al. [63] further reported that the antigen-induced expression CD154 depends on TCR-derived signals, but not on CD28/CTLA-4 costimulatory signal. Further studies suggested that efficient expression of CD154 following polyclonal T lymphocyte activation requires accessory molecules on antigen-presenting cells (APCs), since costimulation of primary murine T cells via CD3 and CD28 can stabilize CD154 expression [64]. Thus, control of CD154 expression involves both costimuli-dependent and -independent pathways [65]. Other mediators of CD154 synthesis include: (i) ionomycin, which induces a very early mRNA and protein surface expression of CD154 within the first 2 h; (ii) the mitogens phytohemagglutinin and concanavalin A, which induce little CD154 itself, but together with PMA, yield markedly elevated CD154 expression [62]; (iii) formyl-methionyl-leucyl-phenylalanine (fMLP), and (iv) physiologically more relevant, proinflammatory cytokines, such as interleukin (IL)-1, TNF-α, and IL-4 [8, 12, 18, 53, 59, 66, 67]. Interestingly, elevated expression of CD154 also occurs after ligation of CD40 [60], indicating autologous regulation of this mediator. Interferon (IFN)-γ as well as transforming growth factor β (TGF-β) has been reported to inhibit CD154 mRNA expression in T lymphocytes [53, 54].

4.2. Regulation of CD154 gene expression

Few studies have addressed the mechanisms underlying CD154 gene expression. Activation of protein kinase C (PKC) and calcineurin, as well as a rise in intracellular calcium concentrations induce CD154 expression [62, 68], a finding supported by the recent demonstration that a calcium ionophore induces transcription of the CD154 gene in T lymphocytes, requiring the calcineurin-dependent transcription factor NF-AT [69]. Reporter gene assays using constructs driven by the promoter of human CD154 together with vectors expressing constitutively active calcium/calmodulin-dependent kinase IV, and calcineurin further revealed synergistic interactions between these two mediators. Finally, a dominant negative mutant of the calcium/calmodulin-dependent kinase IV shows diminished ionomycin-induced activity of the CD154 promoter as well as protein expression. Expression of CD154 can be inhibited by cyclosporin A [68]. Interestingly, patients who received cyclosporin A expressed peripheral blood T lymphocytes deficient in CD154 inducibility [68].

Early molecular characterization of the murine CD154 gene demonstrated a putative site for initiation of mRNA transcription 67 bp upstream of the translation initiation (ATG) codon, consisting of a TATA-like box, an Sp1-like box, and six potential NF-AT-like motifs [26]. Indeed, follow-up studies revealed four NF-AT-binding motifs in the CD154 promoter, consisting of two (cyclosporin A-inhibitable) complexes of NF-ATc and NF-ATp, and further demonstrated that NF-AT proteins are important for the expression of the CD154 gene, whereby transcriptional activity of NF-AT proteins requires AP-1 binding [70]. In addition, work by another group, sequencing a 1.2-kb fragment of the 5′ flanking region of the human CD154 gene promoter, identified two putative binding sites for the NF-AT family of transcriptional activator proteins at -259 bp to -265 bp and -62 bp to -69 with respect to the (cyclosporin A-inhibitable) transcriptional start site [71]. Both binding sites independently modulate CD154 promoter activity in response to T cell activation. Furthermore, two ATTTA elements were identified in the 3′ untranslated region of the murine CD154 gene, probably conferring post-transcriptional stability on the mRNA.

4.3. Regulation of soluble CD154 expression

Recently, soluble forms of CD154 have received more attention, particularly in association with certain human diseases, as reviewed in detail below. The first evidence that activated T lymphocytes not only express cell membrane-associated but also soluble CD154 emerged in 1995 [31]. The kinetics of soluble CD154 expression resemble those observed for the membrane-associated form, though the mechanisms of generation and/or release of soluble CD154 remain poorly understood. Several studies suggested that soluble CD154 is generated by intracellular proteolytic cleavage of the full-length form, producing an 18-kDa fragment starting at methionine 113 that lacks the transmembrane as well as parts of the extracellular domain, but conserves the CD40 ligation domain [31]. Consequently, sCD154 retains the ability to ligate CD40. Whether the proteolytic activity implicated in the formation of sCD154 involves mammalian adamalysins (AMAMs), which belong to the group of metalloproteinases [72], will require future experiments. However, the analogy of CD154 to TNF-α might suggest proteolytic pathways, resembling the function reported for the TNF-α-converting enzyme (TACE) [73, 74].

4.4. Cell types expressing CD154

As indicated above, the synthesis of CD154 was originally thought to be restricted to activated CD4 + T lymphocytes, including cells of the Th0, Th1, and Th2 subtype [8–12]. However, succeeding studies demonstrated that further T lymphocyte subpopulations as well as other leukocytic and non-leukocytic cell types express CD154 (fig. 1). A common feature of the synthesis of CD154 in all cell types is the non-constitutive, inducible expression of this ligand, contrasting with the

mostly constitutive expression of its receptor, as described below.

4.4.1. T lymphocytes. Apart from CD4+ T lymphocytes, further subpopulations are capable of expressing CD154 mRNA and/or protein, including CD8+, CD45RO+/CD45RA+, or Tc1/Tc2 T lymphocyte subsets [53–55, 75, 76], as well as CD4/CD8-negative T lymphocytes [56]. Inducibility of CD154 expression on T lymphocytes seems to depend on maturation, since immature thymocytes do not express CD154 on their surface after stimulation, indicating acquisition of the ability to express the ligand late in thymocyte development [77]. Accordingly, umbilical cord peripheral blood CD4+ T lymphocytes express little or no CD154 upon activation [78].

4.4.2. Basophils. Freshly isolated purified human peripheral blood basophils as well as the human basophilic cell line KU812 express (upon activation) functional CD154, capable of inducing IgE production, suggesting that basophils might play an important role during allergy, not only by producing inflammatory mediators, but also by directly regulating IgE production independently of T lymphocytes [79, 80]. Those findings have been confirmed with umbilical cord basophilic cells [81].

4.4.3. Eosinophils. Peripheral blood eosinophils as well as the eosinophilic cell line EOL-3 express functional CD154 upon activation, whereas eosinophils from hypereosinophilic patients express CD154 constitutively, indicating a role for CD154 in the inflammatory processes involving eosinophil infiltration and activation [82]. Presentation of CD154 at the surface of eosinophils mediates proliferative signals on CD40-positive target cells, such as Reed-Sternberg cells, demonstrating its functionality.

4.4.4. Monocytes/macrophages and Kupffer cells. Initial observations in mononuclear phagocytes revealed CD154 mRNA within monocytes extracted from human peripheral blood [83]. More recent studies demonstrated the inducibility of CD154 mRNA and biologically functional protein in human peripheral blood monocytes in vitro by cytokines, such as IL-1 or TNF-α, and enhanced expression of the ligand on activated monocytes in situ within human atherosclerotic lesions [18] as well as during chronic allograft rejection in human liver allografts [84, 85].

4.4.5. Natural killer cells. Natural killer (NK) cells contain CD154 transcripts [83] and show enhanced CD154 expression upon stimulation with IL-2. The ligand is functional as demonstrated by the killing capability of CD154-positive, but not CD154-deficient, NK cells [86]. These studies suggested a potential role for CD154 in NK cells in immune responses against B cell malignancies.

4.4.6. B lymphocytes. Purified human peripheral blood B lymphocytes, as well as a variety of B lymphoblastoid cell lines and hybridomas can express functional CD154 following activation, which gave rise to the presumption that this mediator might facilitate responses of activated B lymphocytes [87, 88]. Interestingly, B lymphocytes of patients with active systemic lupus erythematosus or hematological malignancies spontaneously express levels of CD154 comparable to those found in T lymphocytes [88–90]. These studies further reported that malignant B lymphocytes coexpressed CD40 and CD154 protein. In vivo studies demonstrated constitutive CD154 expression on peripheral blood B cells in mice, augmented upon endotoxin stimulation [91]. However, mouse B lymphocytes seem to express CD154 in the cytoplasm rather than on the surface, but readily release the mediator as a soluble molecule upon stimulation with anti-Ig antibodies or CD154 itself [33].

In addition, recent studies have established that nonleukocytic cells can also synthesize functional CD154, as described below.

4.4.7. Platelets. Recently, thrombocytes were identified as another source of CD154 [92]. Expression of the ligand was observed only seconds after activation of the platelets in vitro and in the process of thrombus formation in vivo. Further studies demonstrated the biological functionality of the ligand, since it induces expression of chemokines, adhesion molecules, and tissue factor, and diminishes the expression of thrombomodulin in human vascular endothelial cells [93].

4.4.8. Mast cells. The human mast cell line HMC-1, freshly isolated purified human lung mast cells, as well as nasal mast cell from patients with perennial allergic rhinitis express functional CD154 in vitro and in situ [79, 94].

4.4.9. Dendritic cells. Human blood dendritic cells express constitutive CD154 mRNA and protein [60]. Interestingly, ligation of CD40 induces expression of the CD154 gene leading to a rise in ligand levels on the dendritic cell surface [60]. Dendritic cell CD154 is functional, as demonstrated by the finding that CD154-deprived dendritic cells lose their capability to regulate B cell activation and maturation [60]. Lung dendritic cells in mice also express CD154 [95].

4.4.10. Endothelial and smooth muscle cells. Human vascular endothelial and smooth muscle cells express functional CD154 in vitro and at sites of inflammation, e.g., atherosclerotic lesions as well as rejected cardiac and renal allograft transplants, in situ [18, 84, 96, 97]. Cultured endothelial and smooth muscle cells express little constitutive CD154, but show marked increases 12–24 h after stimulation with IL-1, TNF-α, IL-4, or IFN-γ [18, 96].

4.4.11. Epithelial cells. CD154 expression is induced on glomerular and tubular epithelial cells during human

chronic renal allograft rejection [97], but seems to be absent on normal human bronchial epithelial cells [98].

5. Expression of CD40

5.1. Regulation of CD40 protein expression

CD40 was originally described by independent groups who identified an approximately 50-kDa polypeptide on the surface of B lymphocytes or carcinoma cells [1–4]. Signaling via this receptor stimulates the transition of B lymphocytes through the cell cycle, affecting B cell proliferation and DNA synthesis, functions resembling those of a growth factor receptor [99–101]. Originally termed p50 or Bp50 [3, 4], this molecule was initially designated as CDw40 and finally, in 1989, as CD40. This phosphoprotein is characterized by Western blot analysis as three immunoreactive proteins: a main band of 47 kDa, a degradation product of 43 kDa, and a dimer of 85 kDa. Although constitutively expressed on most cell types, expression of the CD40 protein can be regulated. Stimuli for CD40 expression include cytokines, such as IFN-γ, IL-1, IL-3, IL-4, TNF-α [19, 42, 98, 100–105], granulocyte/macrophage colony-stimulating factor (GM-CSF) [105], human immunodeficiency virus (HIV) [106], Epstein-Barr virus latent membrane protein (LMP-1) [107], phorbol esters [108], *Mycobacterium tuberculosis* bacilli [109], 12-O-tetradecanoyl phorbol-13-acetate (TPA) [99], antibodies against IgM or CD20 [99], as well as ultraviolet (UV) light exposure [110]. Induced expression of CD40 is typically observed 6–12 h following stimulation, peaks after 24 h, and persists for an additional 24–72 h, in contrast to the transient expression of its counterpart, CD154.

5.2. Regulation of CD40 gene expression

Very few studies have analyzed the mechanisms underlying CD40 gene activation. Though the CD40 promoter structure is unknown, inducibility of CD40 expression via IFN-γ in most cell types analyzed suggested the presence of signal transducer and activator of transcription (STAT) sites. Indeed, in vascular smooth muscle cells, activation of the CD40 gene via this cytokine is mediated via STAT-1 [111]. Interestingly, CD40 gene expression in these cells via TNF-α is mediated via a different transcription factor, NF-κB [111]. Craxton et al. [112] further demonstrated that potential autocrine induction of CD40 via CD40 ligation does not depend on p38 mitogen-activated protein kinase (MAPK), a mediator of other CD40-mediated functions. These studies further revealed that induction of CD40 expression via proinflammatory cytokines is regulated at the transcriptional level and requires ongoing protein synthesis [113]. Interestingly, inhibition of CD40 expression by certain cytokines, such as TGF-β, occurs via enhanced degradation of CD40 mRNA rather than directly at the transcriptional level [113]

5.3. Cell types expressing CD40

Detailed studies performed with the original source of CD40 showed expression during early B cell development. Embryogenic B lymphocytes already express CD40 [108, 114, 115], consistent with a functional role for CD40 in B cell ontogeny [116, 117]. In addition to its role in early ontogeny, nearly every human adult B lymphocyte expresses CD40 regardless of its function (naïve cell, centroblast, plasmablast, plasma cell, memory cell) or location (bone marrow, tonsil, spleen, primary/secondary follicle) [as reviewed extensively elsewhere see refs 28, 118, 119]. Furthermore, most malignant/leukemic B cell lines express CD40 independently of the degree of maturation of the affected lineage [3, 99, 108, 115, 120–123]. In addition to the cell-associated form of CD40, tonsillar B lymphocytes and transformed B cell lines can release soluble CD40, which binds to CD154 on T lymphocytes [124, 125], implicating sCD40 in the modulation of T cell stimulation. Other leukocytes capable of expressing CD40 are detailed below.

5.3.1. Hematopoetic progenitor cells. Knowledge regarding CD40 expression on progenitor cells is restricted mostly to the B lymphocyte lineage. Acquisition of the CD40 antigen in human B lymphocyte ontogeny occurs subsequent to the expression of CD10 and CD19 antigens but precedes the surface expression of CD20, CD21, CD22, CD24, surface immunoglobulin M (sIgM), and the rearrangement of Ig heavy-chain genes [115, 126]. Analysis of human CD34+ bone marrow and umbilical cord blood cells also showed transient expression of CD40 early in myeloid development [114]. Several studies demonstrated that dendritic Langerhans, CD34+ progenitor cells derived from cord blood actually express functional CD40 at a density higher than that found on B lymphocytes [21, 127]. In addition, CD40-activated cord blood CD34+ progenitors proliferate and differentiate into cells with prominent dendritic cell attributes, such as priming of allogeneic naïve T lymphocytes, indicating that CD40 ligation generates dendritic cells that may prime immune reactions during antigen-driven responses to pathogenic invasion, thus providing a link between hematopoiesis, innate, and adaptive immunity [128].

5.3.2. T lymphocytes. Originally discovered on T lymphocytes of various body compartments of rheumatoid arthritis patients [129], expression of CD40 transcripts and protein localizes to activated CD4+, CD8+, and $CD4^+/CD8^+$ T cell clones as well as TCR+ T lymphocytes [130, 131]. Follow-up studies

revealed functionality of this receptor, since ligation by CD154 induces CD25 and CD154 expression on resting peripheral blood T lymphocytes, CD69 expression on CD3-activated cells, proliferation of activated CD4+ and CD8+ T lymphocytes, and secretion of IFN-γ, TNF-α, and IL-2, implicating CD154 in T lymphocyte activation [132, 133].

5.3.3. Basophils. Basophils enriched from chronic myeloid leukemia blood and dispersed lung tissue as well as human basophil-like cell lines react with antibodies directed against CD40 [134, 135]. This cell type expresses CD40 protein early during differentiation [136].

5.3.4. Eosinophils. Human peripheral blood eosinophils and activated eosinophils from atopic patients express elevated levels of CD40 transcripts and protein. CD40 expressed on eosinophils is functional, as ligation of the receptor enhances eosinophil survival and induces the release of GM-CSF [137]. In vitro CD40 expression in eosinophils is augmented by IgA immune complexes and reduced by IL-10 [137].

5.3.5. Monocytes/macrophages. Primary human peripheral blood monocytes and the monocytic cell line U937 express CD40 mRNA and CD40 cell surface protein following treatment with GM-CSF, IL-3, IFN-γ, or soluble CD23 [23, 138]. In accordance with other leukocytic cell populations, monocytes express CD40 constitutively. Interaction of CD40/CD154 provides a critical trigger for the CD4+ T cell contact-dependent activation of monocytes [139–143]. This interaction might be bidirectional, because the resulting phenotypic changes on monocytes may mediate T cell activation, thus enhancing and/or prolonging inflammatory responses [144]. Furthermore ligation of CD40 on monocytes/macrophages induces IL-12 expression [141, 145], which can in turn induce CD154 expression on the T lymphocyte [146]. Interestingly, disturbed CD40 expression and signaling patterns have been reported for monocytes/macrophages within UV light-exposed skin [147] as well as within the peripheral blood of HIV-1-positive patients [148].

5.3.6. Dendritic cells. Enrichment of human peripheral blood [149], tonsil [150], or dermal dendritic cells [151, 152] revealed the capability of this cell type to express CD40. Ligation of CD40 on human cord blood-derived CD34+ hematopoietic progenitors [128, 153] as well as CD68+ blood cells [154], or adherent peripheral blood monocytes [155] induced their proliferation as well as differentiation into functional dendritic cells, demonstrating functionality of the receptor. CD40 activation induced dendrite development, alterations in the phenotype, as well as elevated expression of MHC class II antigens, CD25, CD58, CD80 (B7-1), CD86 (B7-2), and CD154, cytokines, such as TNF-α, IL-8, IL-10, and IL-12, and chemoattractants, such as macrophage inflammatory protein (MIP)-1α, MIP-1β, and RANTES [21, 60]. Several groups provided evidence for the potential importance of CD40/CD40 ligand-dependent T lymphocyte-dendritic cell interaction, e.g., for the functional development of B cell follicles and adaptive immune responses [105, 156–160; also reviewed in refs 161, 162]. In particular, the role of CD40 ligation for the induction of IL-12 expression has been the focus of several studies. Originally, ligation of the receptor was considered sufficient to induce expression of the cytokine. However, further studies suggested that induction of IL-12 p40 and p75 expression in dendritic cells, via antigen-specific Th1 lymphocytes, which do not differ in either TCR clonotype or CD154 expression, requires IFN-γ as a second signal [163, 164]. Accordingly, Th2 class lymphocytes do not induce, but interestingly inhibit the expression of IL-12. In situ, elevated expression of CD40 has been reported in dendritic cells within non-Hodgkin's lymphomas [165, 166] as well as rheumatoid synovial fluid and synovial tissue [167].

In addition, non-leukocytic cells can also express CD40 (fig. 1).

5.3.7. Epithelial cells. Immunohistochemical analysis revealed CD40 expression on epithelial cells of human nasopharynx, tonsil, and ectocervical tissue, as well as cultured epithelial cells and several epithelial cell lines [168]. The presence of CD40 on diseased and its absence on unaffected epithelium as well as its inducibility by proinflammatory cytokines, e.g., IFN-γ, indicated a role for epithelial CD40 in the development of carcinomas/epithelial neoplasia at sites of chronic inflammation [42], a hypothesis also supported by the later finding that a large majority of nasopharyngeal carcinoma cells expressed CD40 [169]. Further studies of CD40 distribution in the human thymus revealed that cortical and medullar thymic epithelial cells express this receptor in situ and, inducible via proinflammatory cytokines, e.g., IL-1, TNF-α, or IFN-γ, also in vitro [19, 170]. Further studies demonstrated that CD40 expressed on these thymic epithelial cells provides costimulation for clonal expansion of CD4+ thymocytes [17]. In addition to neoplastic and thymic epithelial cells, normal human bronchial epithelial cells constitutively express CD40 in situ [98]. Interestingly, as in the case of dendritic cells, murine epithelial cells are suggested to express different isoforms of CD40 [50].

5.3.8. Endothelial cells. Three different groups independently described in situ and in vitro expression of CD40 on human endothelial cells [20, 22, 24]. All studies demonstrated constitutive basal expression of CD40, which augmented after stimulation with proinflammatory cytokines, such as IL-1, TNF-α, or IFN-γ. Interestingly, a combination of IFN-γ with either IL-1 or

TNF-α acted synergistically, indicating independent pathways for the induction of CD40 gene expression. The receptor on endothelial cells is functional, since CD40 ligation induces expression of adhesion molecules, such as E-selectin, vascular cell adhesion molecule (VCAM)-1, or ICAM-1. Immunohistochemical analysis localized CD40 on endothelial cells of the normal human spleen, thyroid, skin, muscle, kidney, lung, blood vessels, and umbilical cord [20, 22, 24, 172]. However, endothelial cells of tissue involved in inflammatory disease, benign tumors of vascular origin, renal carcinomas, and Kaposi's sarcoma were also demonstrated to express elevated CD40 compared to undiseased tissue [20, 172–174], supporting the hypothesis that this surface receptor plays an important role in tissue inflammation. Further studies revealed that other inflammatory diseases such as allograft rejection [96], atherosclerosis [18, 175], lupus glomerulonephritis [176], HIV infection [106], and probably Alzheimer's disease [177] are associated with elevated expression of endothelial CD40.

5.3.9 Smooth muscle cells. CD40 expression on smooth muscle cells was first described within the atherosclerotic vessel wall [18]. Further studies also demonstrated CD40 expression on cultured smooth muscle cells of the human airways [178]. Both studies showed that CD40 expression on smooth muscle cells is upregulated in vitro upon stimulation with proinflammatory cytokines, e.g., IL-1, TNF-α, or IFN-γ, and that CD40 is functional on these cells [18, 179, 180].

5.3.10. Keratinocytes. Initial studies reported that epidermal keratinocytes express low levels of CD40 in vitro, and had enhanced levels after stimulation with cytokines such as IFN-γ [181]. Later studies demonstrated that undifferentiated and terminally differentiated human keratinocytes express CD40 transcripts as well as protein constitutively [182–184]. The receptor is functional, since ligation induces expression of cytokines, adhesion molecules, and reduces keratinocyte proliferation. Markedly elevated levels of CD40 expression in situ have been reported for the proliferative layers of benign viral-induced cutaneous lesions [185] and in psoriasis, a T cell-mediated inflammatory skin disease [183].

5.3.11. Fibroblasts. Expression of CD40 on human fibroblasts was originally reported by several groups [104, 186, 187], who described CD40 transcripts and protein on human lung, gingival, synovial, dermal, and spleen fibroblasts, as well as on cultured human fibroblasts. Later studies added rheumatoid synovium [188], thyroid fibroblasts in culture [189], orbital connective tissue fibroblasts obtained from normal donors and from patients with severe thyroid-associated ophthalmopathy [190], as well as periodontal ligament and orbital connective tissue fibroblasts [190, 191] to the list of origins. The expression of CD40 mRNA and protein in fibroblasts is induced by proinflammatory cytokines, e.g., IFN-γ [186, 188, 189]. CD40 expressed on fibroblasts is functional and ligation induced expression of adhesion molecules, cytokines, cyclooxygenase-2, and prostaglandin E_2 [104, 192, 193], and inhibited the expression of matrix metalloproteinases (MMPs), such as MMP-1 and MMP-3, in these cells [194]. Furthermore, CD40 expression relates to cell growth, because (i) in vitro confluent fibroblast cultures express higher levels of the receptor than fibroblast in log phase and (ii) ligation of CD40 induces fibroblast proliferation [104, 186]. Further studies suggested that CD40-mediated cognate interactions between tissue fibroblasts and infiltrating T lymphocytes promote fibrogenesis [195].

5.3.12. Carcinomas. Early evidence for overexpression of CD40 in Hodgkin's disease emerged from immunohistochemical analysis [196]. CD40 is strongly expressed with a highly distinct pattern of staining on Reed-Sternberg cells, the presumed malignant cells of Hodgkin's disease, and variants of Hodgkin's disease, irrespective of their antigenic phenotype (T, B, non-T-non-B) or histologic subtype [197–199]. Furthermore, primary and cultured Hodgkin's and Reed-Sternberg cells express high levels of CD40. Functionality of the receptor on these cells was demonstrated by induction of cytokines and adhesion molecules after ligation of the receptor on cultured Hodgkin's and Reed-Sternberg cells [199, 200]. Other, non-lymphoid carcinoma tissue can also express CD40, including epidermal tumors, cutaneous malignant melanoma, osteosarcoma, breast carcinoma, or cervical carcinoma, in which either epithelial cells, endothelial cells, or keratinocytes can express CD40 [169, 172, 185, 201–205].

6. CD40 signaling

Despite the impressive amount of data garnered within recent years, our knowledge regarding CD40-mediated signal transduction pathways and associated transducers remains incomplete and controversial. The controversies result, in part, from differences in CD40-mediated signal transduction among cell types and, furthermore, can vary within the same cell type depending on the stage of differentiation, as previously discussed elsewhere [206]. Most of the signaling studies have employed B lymphocytes, where the analysis of primary cells versus cell lines provided an additional source for controversiality. These considerations should be borne in mind during the following review of the literature analyzing the CD40-mediated signaling pathways.

Based on the structural homologies with the TNF/TNF receptor family, CD154 had been predicted to form trimeric structures, which as a consequence of receptor-ligand interaction results in the trimerization of CD40

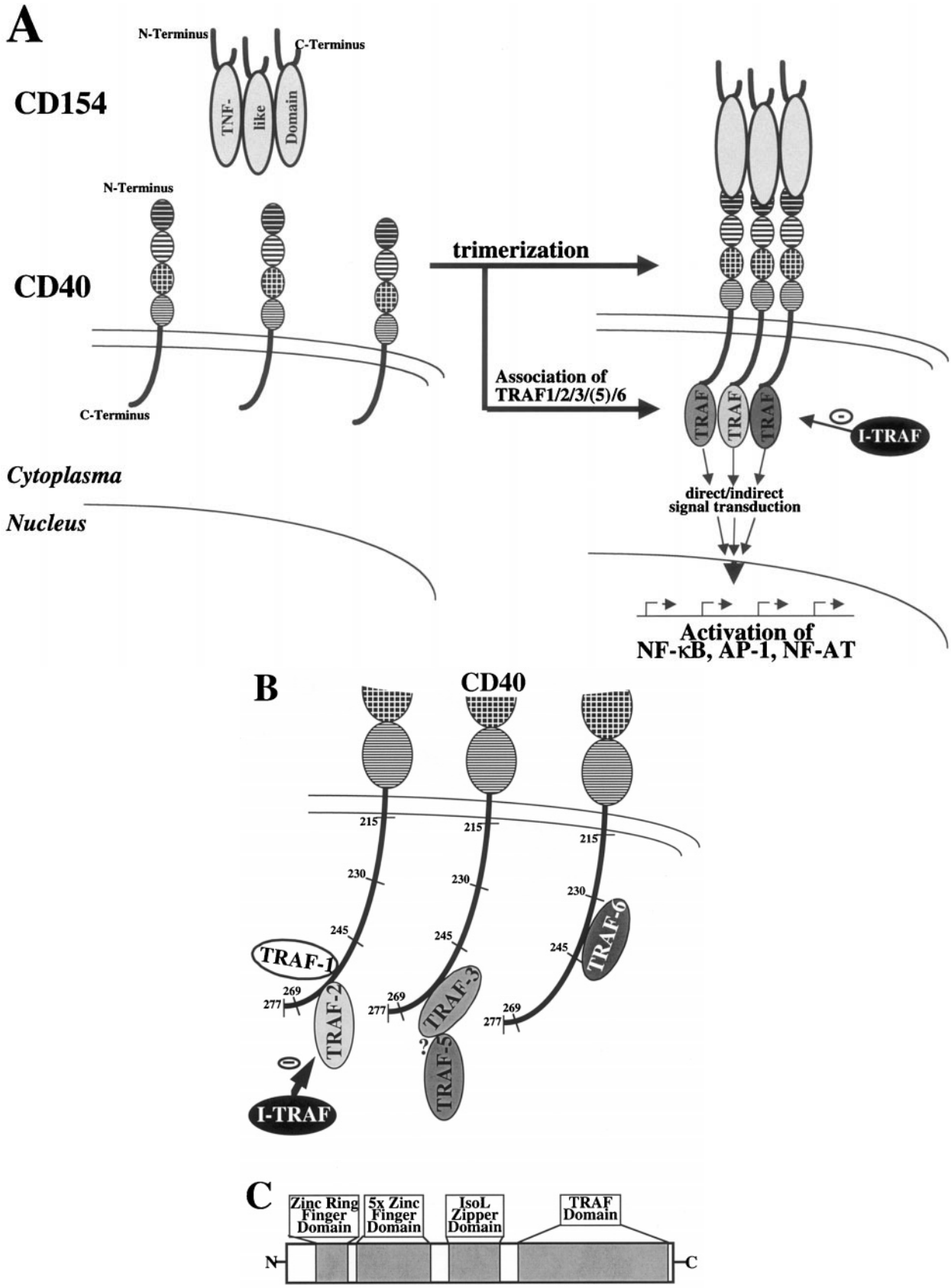

Figure 3. Potential mechanisms of CD40 signaling. Shown are potential extracellular (*A*) and intracellular (*B*) processes occurring during CD40 signaling involving the respective members of the TRAF family. (*C*) Prototypical organization of TRAF proteins.

receptor proteins [37] (fig. 3A). Indeed, CD154 and subsequent CD40 oligomerization are crucial steps in CD40-mediated signal transduction; hence, trimeric CD154 molecules exhibit the higher potency compared to monomeric or dimeric forms [207].

Initial studies on CD40 signaling focused on the phosphorylation of the receptor itself [101, 208]. CD40 contains at least two major signaling determinants in the cytoplasmic domain, one of which includes threonine 234 [46]. Substitution of an Ala for this Thr at position 234 as well as deletion mutants lacking Thr234 inhibit signal transduction after CD40 ligation [209]. Furthermore, these phosphorylation sites reside within areas of the cytoplasmic domain deemed critical for signal transduction because of association with CD40-binding proteins [210], as discussed below. These findings, in combination with the lack of intrinsic protein kinase activities [10] of the receptor, suggested the following events in CD40-mediated signal transduction: (i) activation of separate kinases/phosphatases and/or (ii) association of regions of the cytoplasmic domain with binding proteins. As discussed in detail below, both mechanisms probably contribute to the CD40-signaling pathway.

6.1. Activation of kinases/phosphatases

Most studies implicate activation of protein tyrosine kinases and protein tyrosine phosphatases as immediate intracellular responses during CD40 activation. Initial studies demonstrated that ligation of CD40 on activated, but not on resting mature B lymphocytes enhanced tyrosine phosphorylation of four distinct phosphoproteins with molecular masses of 67, 72, 96, and 113 kDa, and induced a rapid increase in the production of inositol 1,4,5-trisphosphate [211]. The same study further identified five electrophoretically distinct renaturable, CD40-regulated serine/threonine-specific protein kinases (PK120, PK93, PK76, PK55, and PK48) that showed markedly increased in vitro activity after CD40 stimulation. Furthermore, CD40 ligation causes phosphorylation of phospholipase Cγ2, and the 85-kDa, but not the 110-kDa, subunit of the phosphatidylinositol-3-kinase, increasing their activity, and suggesting a role for these two enzymes in CD40 signal transduction [212]. These findings agree with later reports describing rapid induction of a wortmannin (or similar inhibitor)-sensitive kinase activity, probably phosphatidylinositol-3-kinase, following CD40 ligation [213]. Furthermore, the finding that stimulation of Daudi B cells with an activating anti-CD40 antibody stimulates p21ras, in parallel with tyrosine phosphorylation of phosphatidylinositol 3-kinase and stimulation of Rac1 and MEK-1 [214], implicated participation of the Ras pathway in CD40 signaling.

Whether CD40-mediated signal transduction pathways involve PKA, e.g., during B cell activation, remains controversial [215–217]. Cyclic AMP can increase following CD40 ligation and regulate CD40 signaling either positively or negatively [216, 217]. The role of PKC in CD40 signaling seems restricted to downstream rather than immediate signaling events [211, 212, 218–223]. Ligation of CD40, for example, transiently activates stress-activated protein kinases (SAPK) via PKC-independent pathways [224]. Some studies reported that CD40 ligation does not affect members of the MAPK family, e.g., extracellular signal-regulated protein kinase (ERK)-1 and ERK-2, while others describe involvement of the ERK cascade, in particular ERK-2, in CD40 signaling in a mouse B cell lymphoma cell line [225, 226]. Other studies report that CD40 engagement enhances ERK activities [227, 228]. Again, these disparate results may reflect use of cells of different origin as also indicated by a recent study demonstrating that CD40 ligation induces ERK activation in normal B lymphocytes, but not or only weakly in B lymphocyte lymphoma cells (WEHI-231) [215]. In monocytes, ligation of CD40 resulted in the phosphorylation and activation of ERK-1 and ERK-2, but not phosphorylation of other MAPK family members, such as p38 or c-Jun N-terminal kinase [229].

Studies in which protein tyrosine kinase inhibitors, such as herbimycin, block aggregation as well as rescue of germinal B lymphocytes, indicated the potential importance of protein tyrosine kinases in the pathway of CD40 signaling [230, 231]. In particular, the Src family tyrosine kinases, syk, lyn, and fyk, have been associated with CD40 signaling [212, 232]. Ligation of CD40 finally also induces alterations in the state of tyrosine phosphorylation of CD45, a protein tyrosine phosphatase [232].

6.2. CD40-binding proteins

Since direct association of the respective kinases/phosphatases with CD40 could not be demonstrated, the association of intermediary CD40-binding proteins has been considered crucial in CD40-mediated signal transduction, possibly ultimately mediating the more downstream function of those non-receptor kinases/phosphatases described above. In 1994, a member of the family TNF receptor-associated proteins, TRAF-3, was identified as the first protein associated with the cytoplasmic domain of CD40. Originally, this ubiquitously expressed 62-kDa intracellular protein had been termed CD40-binding protein [210], CRAF1 [CD40-receptor associated factor 1; 233], LAP1 [LMP-1-associated protein 1; 234], or CAP1 [CD40-associated protein 1; 235]. A direct role for TRAF-3 in CD40 signaling was postulated after the demonstration that the growth

transformation of B lymphocytes by Epstein-Barr virus is mediated through the interaction of the carboxy-terminal 44 amino acids of the virus latent infection membrane protein 1 (LMP-1), which contains various TRAF-binding sites [234, 236]. TRAF-3, located on chromosome 14, band q32.3, is encoded by a single gene comprised of 13 exons, spanning 130 kb, that generates a variety of mRNA species by alternative polyadenylation, mRNA splicing, and transcription initiation [237]. TRAF-3 interacts directly with the cytoplasmic tail of CD40 through a region termed the TRAF domain [209, 233, 235]. This domain is required for association with the cytoplasmic domain of the related 75-kDa TNF receptor and can form homo- and heterodimers.

The TRAF family consists of six known members (TRAF-1 to TRAF-6) that share homology in the C-terminal TRAF domain, required for multimerization and binding to members of the TNF receptor family (see also fig. 3). Following TRAF-3, another member of the family, TRAF-2, has been shown to bind to the cytoplasmic domain of CD40 [238]. Recent data indicate that the association with CD40 might be regulated via the phosphorylation status of TRAF-2 [239]. In addition to the CD40-binding domain, both TRAF members share several other functional domains: the N-terminal portion of TRAF-3 and TRAF-2 contains a RING finger motif and five zinc finger-like domains similar to those found in DNA-binding proteins [210, 233, 235, 238]. Based on the structural similarities within the putative DNA-binding domains, TRAFs have been speculated to function as direct transcriptional regulators [206]. DNA binding of the CD40-associated factors would require release from the receptor. Even though CD40 and TRAF-2 were found constitutively associated with each other, ligation of CD40 itself seems to inhibit the binding of TRAF-2 to the CD40 cytoplasmic domain [240]. However, final evidence for a direct path from CD40 ligation to DNA regulatory functions via TRAFs remains to be demonstrated. Moreover, binding of TRAFs via their conserved TRAF domain to the respective receptor might be further regulated by TRAF-interacting protein(s), endogenous inhibitors of TRAF functions, such as the recently described I-TRAF, which inhibits TRAF-2-mediated NF-κB activation signaled by CD40 [241].

Following TRAF-2 and TRAF-3, further members of the TRAF family, TRAF-5 and TRAF-6, have been associated with CD40 signaling [242, 243]. Both TRAF-5 and TRAF-6 contain the characteristic carboxy-terminal TRAF domain and the amino-terminal RING finger domain as well as a cluster of zinc fingers. Overexpression of TRAF-5 and TRAF-6, in contrast to TRAF-3, activates NF-κB. TRAF-6, which associates with CD40, but interestingly not with the cytoplasmic tails of TNF receptor type 2 [243, 244], further activates ERK [227], indicating only one of several possible links to the kinase/phosphatase pathways described above. Whether TRAF-5 associates with the cytoplasmic tail of CD40, as suggested by Ishida et al. [242] is controversial, since other groups demonstrated that TRAF-5 binds to the cytoplasmic region of the lymphotoxin-β receptor, but not to most nerve growth factor receptor family members, including CD40 [244, 245].

Using peptides with progressive deletions, the PVQET as well as the QEPQEINF sequence within the CD40 cytoplasmic domain were mapped as the minimal TRAF-1-, TRAF-2-, and TRAF-3-, as well as TRAF-6-binding region, respectively, indicating that the CD40 cytoplasmic domain contains two non-overlapping TRAF-binding regions, associated with residues 203–245 and residues 246–269, respectively (fig. 2) [242–244]. Interestingly, the TRAF domains of TRAF-1, TRAF-2, TRAF-3, and TRAF-6 formed homotrimers in solution, suggesting that TRAF trimerization is required for high-affinity interactions with CD40 [246]. Accordingly, monomeric TRAF-C domains bind less potently to CD40 than trimeric TRAFs. With respect to binding affinities, trimeric TRAF-2 was stronger than that of TRAF-3. Although TRAF-1 and TRAF-6 also bind to CD40, their affinities were found to be much less. The composition of TRAFs within a cell probably varies with the activation status, since CD40 ligation on B lymphocytes recruits TRAF-2 and TRAF-3 to the receptor, while suppressing other, non-receptor-associated TRAFs.

In summary, the following scenario emerges from these data: binding of the trimeric ligand causes trimerization of CD40, a process that presumably allows the trimeric association of members of the TRAF family, particularly TRAF-2 and TRAF-3, and probably less efficiently TRAF-1 and TRAF-6 (as well as probably TRAF-5 via association with TRAF-3) with the receptor (as summarized in fig. 3A,B). Depending on the cell type, certain TRAF members might preferentially associate with the CD40 cytoplasmic domain and mediate downregulation of other, non-associated TRAFs. These CD40-binding proteins finally, directly and/or indirectly, might regulate gene transcription, probably in part via activation of NF-κB or AP-1.

The hypothesis that TRAF proteins couple CD40 to the kinase cascade, which finally might activate NF-κB, JNK, and/or p38 MAPK, receives further support from the demonstration that the cytoplasmic domain sequence, which is required for the binding of TRAF-1, TRAF-2, and TRAF-3/TRAF-5, constitutes an independent signaling motif, reported sufficient for the activation of JNK and p38 MAPK pathways, as well as for IκBα phosphorylation and degradation [247]. The hypothesis of coexisting direct and indirect TRAF-medi-

ated gene transcription is also supported by the demonstration that different TRAFs as well as different domains of the respective TRAFs might mediate NF-κB and JNK signaling differentially [248–250]. TRAF-2, TRAF-5, and TRAF-6 effectively initiate NF-κB activation (a function that requires an intact zinc ring finger) as well as the JNK pathway, whereas TRAF-3 initiates independent signaling pathways via p38 and JNK, but does not affect NF-κB activity. Furthermore, different TRAF family members might activate the same transcription factor, e.g., NF-κB, by separate pathways [250]. In accordance with this finding, Kehry's group demonstrated that optimal NF-κB and JNK activation requires both TRAF-1/TRAF-2/TRAF-3- and TRAF-6-binding sites, whereas p38 MAPK activation depends primarily on TRAF-6. These results suggest a role in CD40 signaling for competitive TRAF binding and imply that CD40 responses reflect an integration of signals from individual TRAFs [251]. Furthermore, evidence has been provided that different target genes might be regulated via different TRAF family members. CD40 mutants incapable of binding certain TRAF members demonstrated that TRAF-2 rather than TRAF-3 is critical for the gene transcription of ICAM-1, which is probably NF-κB mediated [252]. These findings agree with the earlier association of TRAF-2 rather than TRAF-3 with NF-κB-mediated gene activation [238].

The involvement of other proteins in CD40 signaling remains to be determined. Recently, a novel 61-kDa serine/threonine kinase has been identified as part of the CD40 signaling complex, and termed receptor-interacting protein 2 (RIP-2); it interacts with TRAF-1, TRAF-5, and TRAF-6, but not with TRAF-2, TRAF-3, or TRAF-4 [253]. Furthermore, a recently identified member of the TNF receptor superfamily termed receptor activator of NF-κB (RANK) contains two distinct domains in the cytoplasmic tail that provide multiple TRAF-binding sites, mediating the association with TRAF-1, TRAF-2, TRAF-3, TRAF-5, and TRAF-6, as well as the induction of NF-κB, AP-1, and c-Jun NH_2-terminal kinase activities [254].

6.3. The role of STATs

Besides TRAFs, members of the STAT family were originally associated with CD40 signaling, probably via mechanisms resembling those described above for the TRAF pathway. STAT proteins, which become phosphorylated by kinases of the Jak family after ligation of the IFN-γ receptor, assemble into complexes, translocate to the nucleus, and bind specific DNA elements, enabling them to direct transcription [255]. In accordance with this hypothesis, CD40 engagement on murine B cells results in rapid tyrosine phosphorylation of the STAT-6 transcription factor, transactivation of a reporter gene containing an IFN-regulatory factor-1 STAT-binding site [256], and the expression of members of the Janus family of protein tyrosine kinases, such as Jak-3 [257]. However, recent studies suggested that, at least within B lymphocytes, JAK-3 is not essential for CD40 signaling [258]. Hence, the role of STAT-6 in CD40 signaling remains unclear, since mutation of a STAT-6-binding site in B lymphocytes did not affect transcription of the lymphotoxin α gene driven by stimulation with an anti-CD40 antibody [259]. Other studies demonstrated that ligation of CD40 indeed induces STAT-6, but further demonstrated that a STAT-6-binding site might not be required for the transcription of target genes [260].

6.4. Transcription factors

Regarding the transcription factors involved in CD40-mediated signaling, early studies already demonstrated that ligation of CD40 activates NF-κB and NF-κB-like transcription factors, including the NF-κB family members p50, relA, and c-Rel [218, 261, 262]. Kosaka et al. [263] recently showed that ligation of CD40 results in the activation of IκB kinase in a human B cell line, and furthermore established a functional link between TRAF-2 and IκB kinase activity. CD40 ligation has also been suggested to induce NF-κB expression via the PI3-kinase pathway [219]. Recent studies in WEHI cells demonstrated that a 17-amino acid sequence within the cytoplasmic domain, termed CD40 TNF-associated factor family member-interacting motif (TIM), confers NF-κB activability [264]. CD40-mediated NF-κB activation might require IL-4 as a cofactor, at least in certain cell types [265].

Aside from NF-κB, CD40 ligation results in the activation of the inducible transcription factor complex, AP-1 [218], typically consisting of heterodimers between Jun and Fos proteins, a finding consistent with the description of CD40-induced expression of JunB, JunD, and c-Fos in primary B lymphocytes [266]. However, these data conflict with results of another study describing CD40 signal upregulation of c-jun but not c-fos mRNA in primary B lymphocytes as well as B cell lines [224]. The latter study also provided evidence that CD40 signals alter the transcription factor ATF2 but not the Raf-1 protein. Furthermore, ligation of CD40 induces activation of NF-AT in humans [218, 267] as well as in mice [268], and modulates E2F activity in B cell lymphoma [269]. The CD40-dependent activation of these members is rapid, and evidence has been provided that it is mediated through a tyrosine kinase- and TRAF-dependent pathway [262]. Reactive oxygen intermediates have also been suggested to act as second messengers during CD40 signaling [270].

Furthermore, some evidence supports convergence of the signaling pathways for CD40 and the B cell receptor. It remains to be determined, however, whether this occurs within the cytosol and is mediated via kinases, such as those of the Src kinase family [206], or the MAPK kinase, MEK-1 [215], or within the nucleus, mediated via respective transcription factors [218].

The studies reviewed above address mainly CD40 signaling pathways in B lymphocytes. Only recently have studies also investigated mechanisms involved in CD40 signaling in other cell types. Within monocytes, CD40 ligation results in the phosphorylation and activation of the MAPKs ERK-1/2. In fibroblasts, CD40 signaling mediated NF-κB mobilization [189, 190, 274]; within epithelial cells, CD40 ligation induced growth in a TRAF-3-dependent manner [272]. In smooth muscle cells, cross-linking of CD40 resulted in increased intracellular calcium concentrations, activation of protein tyrosine kinases, protein tyrosine phosphorylation, and activation of NF-κB [178].

Future studies will have to determine whether employment of TRAF family members reflects the (major) CD40 signaling pathway, whether these CD40-binding proteins interact with DNA directly or via other messengers/transcription factors, and whether differential equipment of certain cell types with TRAF family members, or differential use thereof, accounts for the diversity in responses to CD40 ligation.

7. Biological function of the CD40/CD154 dyad

Since numerous studies have addressed the role of CD40 signaling in humoral immunity and various excellent reviews have documented these findings, we will address this issue only briefly and refer to previous publications for further detail [28, 118, 119, 273–279].

7.1. T cell-dependent humoral immunity

The interaction between CD40 and CD154 is crucial for primary and secondary thymus-dependent humoral immune responses, and for the maturation towards memory B lymphocytes. Beside the mitogenic function, CD40/CD154 interactions further regulate the expression of costimulatory factors. Activation of naïve T lymphocytes requires both: the antigen-specific signaling via engagement of the TCR and the signaling via costimulatory molecules, such as the CD80/CD86 system, which interacts with CD28 on T lymphocytes. The first signaling pathway induces the expression of CD154 on the T lymphocyte, which via direct interactions can augment levels of costimulatory factors such as CD80/CD86 on B lymphocytes or other APCs, which in turn can amplify the activation of the T lymphocyte. Such activated T lymphocytes can initiate the secondary (humoral or cellular immune) response by regulating the activation and proliferation of naïve and mature B lymphocytes, switching of immunoglobulin classes, rescue from apoptosis, formation of germinal center cells, and the maturation toward memory cells.

7.1.1. Activation of B lymphocytes. Initial studies reported that ligation of CD40 via recombinant CD154 or anti-CD40 antibodies on human B lymphocytes elevated cell volume [100, 102], a process that correlated with the induction of intracellular, cell surface, and soluble markers, such as adhesion molecules, causing homotypic aggregation of freshly isolated human B cells via ICAM/LFA-1 [100, 230, 280], as well as VLA-4-dependent adhesion of B lymphocytes to endothelium [281]. CD40 ligation furthermore elevates the expression of other cell surface markers, including the low-affinity IgE receptor CD23 [57, 117], CD30 [282], CD80 (B7.1) and CD86 (B7.2) [63, 283–285], Fas [286, 287], and MHC class II [273]. In addition, CD40 ligation triggers the expression of the soluble cytokines IL-6, IL-10, TNF-α, TNF-β, and TGF-β [208, 288–290]. In contrast, CD40 ligation reduces the expression of CD8 [291]. Although the function of several of these mediators remains to be determined, most studies implicate CD40/CD154 interactions in modulation of B lymphocyte growth and differentiation.

The induction of B lymphocyte proliferation was the original function employed to isolate the first anti-CD40 antibodies. Resting as well as activated B lymphocyte subpopulations, including naïve, memory, germinal center CD5 − and CD5 + B lymphocytes respond to CD40 ligation with enhanced proliferation rates [292, 293]. However, CD40 ligation supports only limited, short-term (< 10 days) proliferation. Long-term proliferation requires a costimulatory signal, e.g., via multivalent immunoglobulin cross-linking [294, 295] or cytokines, such as IL-4 and IL-10 [8, 11, 116, 292, 296–299]. Interestingly, IFN-α significantly inhibited CD40-mediated proliferation of normal tonsillar B lymphocytes [300].

In addition to NF-κB [301], control of B lymphocyte proliferation may involve the phosphoinositide 3-kinase subunit p85α and its splice variants p55α and p50α. Mice genetically deficient for these proteins show diminished numbers of peripheral mature B lymphocytes and proliferative response to CD40 ligation, a phenotype resembling observations in mice deficient in the tyrosine kinase Btk [302].

7.1.2. Switching of immunoglobulin classes. Probably the most intensely studied biological function of CD40 ligation on human B lymphocytes is the switch in recombination and synthesis of immunoglobulins, the prevalent mediators of this leukocyte subpopulation. CD40 signaling is essential for thymus-dependent induction of

immunoglobulin isotype synthesis itself as well as for the switching of the isotype of the heavy chain. Despite the impressive number of published studies, whether CD40 signaling (without further costimuli) is sufficient to mediate isotype switching remains controversial. Although some studies demonstrated that engagement of CD40 in the absence of other stimuli suffices to induce switching to IgG or IgA in human B cells [288, 303], several studies indicated that switching to at least certain immunoglobulin classes might require, or be potentiated by, the presence of certain comediators, e.g., T helper cell-derived cytokines, such as IL-4 or IL-10 [304–309]. Further studies also suggested that bidirectional modulation of immunoglobulin synthesis via cytokines: IL-2 and IL-10 specifically enhance IgM, IgG1, and IgA production, whereas IL-4, despite costimulating B lymphocyte proliferation, does not augment secretion of these isotypes, but rather provides an essential cosignal with CD154 for the production of IgG4 and IgE [310]. Cosignaling via cytokines has also been implicated in CD40-mediated IgD production; however, few data exist regarding synthesis of this immunoglobulin class, as reviewed elsewhere [118].

The dependence on CD40 signaling in combination with the requirement for comediators has been investigated in some detail for the synthesis of the primary immunoglobulin isotype generated during the T cell-dependent humoral response, IgM. Although probably predominantly mediated by CD40 (anti-CD40 treatment completely abolishes the primary IgM response in vivo [13]), IgM synthesis is potentiated in the presence of IL-2, IL-4, IL-5, and/or IL-10 [298, 310–312].

Induction of IgE expression seems to require both CD40- and IL-4-mediated signaling, which can be further enhanced by IL-5, IL-6, and/or IL-10, probably involving an autocrine feedback loop [298, 306, 310–316]. In addition, IL-13, which has high homology to IL-4, can support IgE synthesis, probably independently of IL-4 [317]. Regarding the immunoglobulin switch towards IgE, molecular mechanisms have been discovered that suggest that cytokines, such as IL-4, modulate isotype switching via the activation of certain DNA-binding proteins, e.g., an IL-4 nuclear factor interacting with the IL-4-responsive element in the ε switch region, thus inducing Cε germline transcript expression as well as class switching to IgE [315, 318]. This Cε germline transcription and switch recombination is opposed by IFN-γ and IFN-α, TGF-β, retinoic acid, and IL-6 [319–323].

Regarding the synergistic interaction of CD40 and IL-4 on the production of immunoglobulins, recent studies have proposed the formation of additional, STAT-6- and NF-κB/Rel protein-containing nuclear complexes. These complexes bind to the CD154/IL-4-responsive region of the respective promoter and thus enhance the activation via CD40 ligation, which mediates binding of NF-κB/Rel proteins to two tandem κB sites [324]. Similarly, studies have identified an evolutionary conserved sequence upstream of the human Ig heavy-chain Sγ3 region that acts as an inducible promoter, containing cis elements that critically mediate CD154- and IL-4-triggered transcriptional activation of the human Cγ3 gene [260]. This sequence provides a tandem NF-κB/Rel-binding motif, which is critical for the responsiveness to both CD154 and IL-4. However, activation via IL-4 requires an additional STAT-6-binding site. Further studies demonstrated that the transcription factor B cell-specific activator protein (BSAP) might be the merging point of the two signaling pathways, since it enhances both IL-4- and CD154-mediated activation of the human ε germline promoter [325]. Interestingly, CD40-mediated differentiation of non-antigen-selected human B cells is critically regulated by CD30, which inhibits the Ig switching, possibly through interference with the CD40-mediated, NF-κB-dependent, transcriptional activation of downstream C(H) genes [282].

7.1.3. Formation of germinal center and memory cells. The CD40 and CD154 receptor-ligand dyad affect the formation and survival of germinal center as well as memory B cells. One of the first functions implicating the CD40/CD154 dyad in the formation of germinal centers was the achievement of survival of freshly isolated centrocytes via CD40 ligation, as reviewed in detail elsewhere [326, 327]. CD40 signaling on germinal center cells furthermore increases the expression of surface immunoglobulin, and shifts their development towards memory cells. In accordance with this hypothesis are further studies indicating that CD40 ligation plays an indirect rather than a direct role in germinal center formation, since CD40 signaling bidirectionally activates T lymphocytes to induce secretion of cytokines required for germinal center formation and B lymphocytes to express the respective cytokine receptors [328]. Within advanced germinal centers, the bidirectional activation might also prime B lymphocytes for evolution toward memory cells [329]. Arpin et al. [293] demonstrated that prolonged stimulation of CD40 signaling on germinal center B lymphocytes in the presence of IL-2 and IL-10 mediates differentiation into memory cells, whereas lack of the ligand in this system results in differentiation toward plasma cells.

7.1.4. Activation of T lymphocytes via CD154. Although signaling following ligation of CD40 by CD154 was originally considered restricted to B lymphocytes, several studies demonstrated that cross-linking of CD154 on T lymphocytes provides costimulatory signals in the development of T helper responses. In vitro studies demonstrated that induction of IL-4 expression via CD3 and/or CD28 requires costimulation of T cells with CD154 [330]. In accordance with the role of IL-4 in the development of T helper cells, in vivo studies revealed that costimula-

Table 2. Biological functions of CD40 ligation.

	Function	Cell type	Mediator
Humoral immunity	activation	B lymphocytes	CD23, CD30, CD80, CD86, Fas, MHCII; cytokines: IL-6, IL-10, TNB-α, TGF-β
	Ig class switching	B lymphocytes	IgA, IgD (?), IgE, IgG, IgM
	Formation of germinal center and memory cells	B lymphocytes	cytokines: IL-2, IL-10; cytokine receptors
Cellular immunity	pro-inflammatory cytokines	B lymphocytes eosinophils monocytes dendritic cells epithelial cells fibroblasts keratinocytes endothelial cells smooth muscle cells	IL-1, IL-2, IL-4, IL-8, IL-10, IL-12, TNF-α, TGF-β
	chemokines	monocytes epithelial cells fibroblasts keratinocytes endothelial cells	IL-8, MIP-1α, MIP-1β, RANTES, MCP-1 ABCD-1: CCR7 (diminished CCR1/5)
	adhesion molecules	B lymphocytes endothelial cells fibroblasts	LFA-1, ICAM-1, VCAM-1, E-selectin, VLA-4
	matrix metalloproteinases	monocytes fibroblasts endothelial cells smooth muscle cells	interstitial collagenase 1 and 3 (MMP-1, MMP-13), gelatinase A and B (MMP-2,MMP-9), stromelysin 1 (MMP-3) and 3 (MMP-11)
	procoagulant activites	monocytes endothelial cells smooth muscle cells	tissue factor
	others	monocytes dendritic cells fibroblasts endothelial cells	Cox-2, nitric oxide

tion of T lymphocytes via CD154 is associated with the differentiation into T helper cells, modulating humoral immune responses of B lymphocytes [331].

7.2. Regulation of inflammatory mediators

Originally considered restricted to thymus-dependent humoral immunity, CD40/CD154 interactions might also participate in the development of cell-mediated immune responses. Substantial data now implicate CD40 signaling in the priming as well as effector functions of T lymphocytes. Ligation of CD40 activates proinflammatory processes in other leukocytic as well as non-leukocytic cells, including macrophages, NK cells, endothelial and smooth muscle cells, keratinocytes, and fibroblasts, as outlined in table 2 and discussed in detail below.

7.2.1 Cytokines. The cytokine network connects numerous immune and inflammatory processes involving leukocytic as well as non-leukocytic cell types. Ligation of CD40 on normal human blood B lymphocytes can stimulate the synthesis of IL-2, IL-6, IL-10, TNF-α, lymphotoxin α, and TGF-β [208, 288–290, 332–334]. Among the first indications of functional CD40 on cell types other than B lymphocytes was the report on the induction of GM-CSF expression [19], originally observed in thymic epithelial cells, and later also reported in follicular dendritic cells [335] and eosinophils [137]. Originally thought to require cytokines as costimulators [23], ligation of CD40 alone was eventually found to trigger expression of cytokines, such as TNF-α, IL-1, IL-6, or IL-8, in peripheral blood monocytes [144]. The CD40-mediated induction of IL-1β and TNF-α may follow activation of the MEK/ERK pathway, which is

antagonized by signals generated through the action of IL-4 and IL-10 [229]. Independent groups reported similar observations, with the exception of TNF-α, in human vascular endothelial cells [22]. CD40-mediated IL-6 production, mostly associated with enhanced proliferation of the respective cell type, has been described in multiple myeloma cells [336], in synovial membrane and dermal fibroblasts [104, 191], in keratinocytes [182], and in other non-hematopoetic cells [271]. CD40 ligation-induced IL-8 expression in fibroblasts [190], macrophages, or dendritic cells has been associated with the production of active IL-12 in these APCs [141, 337, 338], a process occurring during responses to T cell-dependent antigens. Interestingly, IL-12 can augment CD154 expression on T lymphocytes, indicating potential paracrine activation of the CD40/CD154 and/or IL-12 pathway [146]. The CD40-induced expression of IL-12 probably results from activation of NF-κB [339], and is inhibited in the presence of IL-10 and, less efficiently, also by IL-4, a finding associated with the processes resulting in priming of a Th1/Th2 response [340]. Furthermore, CD40-induced IL-12 may regulate IFN-γ production in peripheral blood monocytes [341]. However, whether CD40/CD154 interactions are essential for the induction of these Th1 immune response mediators remains to be determined, since the expression of both IL-12 and IFN-γ in CD154-deficient mice did not differ from that in wild-type mice [342].

7.2.2. Chemokines. Although the induction of IL-8 in mononuclear phagocytes was reported several years ago [23, 144], CD40-mediated induction of chemotactic mediators has only recently attracted interest. CD40 ligation induces expression of IL-8 in keratinocytes [183], fibroblasts [195], and epithelial cells [343]. Moreover, CD40 signaling also induces MCP-1, RANTES, MIP-1α and MIP-1β expression in proximal tubular epithelial cells [343], endothelial cells [92], and macrophages and macrophage-derived dendritic cells [344, 345]. Recently, ABCD-1 was identified as a CD40-inducible chemokine in B lymphocytes. This mediator is thought to play an important role in the collaboration of dendritic cells and B lymphocytes with T cells during immune responses [346]. Some groups have reported that the CD40-mediated expression of chemokines is enhanced by costimulation with cytokines such as IL-4, IL-13 [347], or IFN-γ [205]. Interestingly, ligation of CD40 does not necessarily result in the upregulation of chemokines: CD40 ligation, while stimulating the production of the other chemokines, caused a pronounced reduction of myeloid progenitor inhibitory factor 1 (MPIF-1) on human dendritic cells [348]. Moreover, recent reports demonstrated that chemokine receptors might be regulated differentially: maturation of dendritic cells by CD154 induces rapid downregulation of the CC chemokine receptors CDR-1 and CCR-5, but slow upregulation in the expression of CCR-7 [345, 349, 350].

7.2.3. Adhesion molecules. The induction of adhesion molecule expression was among the first biological consequences reported for CD40/CD154 interactions. Original reports, however, focused on its function in humoral rather than cellular immunity. Several studies demonstrated that CD40 signaling induces CD18/ICAM-1 (CD54)- and CD11a/CD18 (LFA-1)-mediated adhesion in B lymphocytes [230, 280], which implicates IgE synthesis by modulating Cε germline transcription [351]. Further studies revealed that CD40/CD154 interactions also mediate heterotopic adhesion. Treatment with an activating anti-CD40 antibody induced the heterotypic adhesion of normal B lymphocytes and B cells lines to both untreated and IL-1-stimulated endothelial cells via the interaction between the very late antigen 4 (VLA-4) and VCAM-1 [281]. In 1995, several groups transferred the idea of CD40-induced adhesion molecule expression into cellular immunity: a trimeric form of recombinant murine CD40 ligand induced the expression of leukocyte adhesion molecules, such as E-selectin, VCAM-1, and ICAM-1 on human vascular and umbilical vein endothelial cells [20, 22, 24], as well as on synovial membrane or dermal fibroblasts [104]. Furthermore, CD40 ligation on HIV-infected endothelial cells stimulated the preferential induction of VCAM-1, thus promoting B lymphoma cell growth through attachment, indicating a potential pathway through which viruses may induce malignancies [106]. These findings suggested the hypothesis that endothelial CD40 might serve as a crucial signaling receptor in the development of T lymphocyte-mediated (chronic) inflammatory reactions. Kinetic studies demonstrated that CD40/CD154-mediated activation of endothelial cells follows the time course of adhesion molecule induction observed with IL-1 or TNF-α, inducing peak levels of E-selectin after 4 h and of ICAM-1 after 24 h of stimulation [352]. CD40-mediated expression of adhesion molecules has further been described for CD54 on dendritic cells [338] and keratinocytes [183]. Later studies associated CD40-mediated expression of adhesion molecules with pathogenic processes underlying prevalent human diseases. Reed-Sternberg cells, regarded as the malignant components of Hodgkin's disease, as well as myeloma cell lines upregulate expression of adhesion molecules, such as CD54, LFA-1, or ICAM-2, upon CD40 ligation [353, 354]. Recent studies revealed that CD40 ligation induces the expression of the 86-kDa subunit of the Ku autoantigen, which functions as an adhesion molecule and mediates homotypic and heterotypic adhesion of multiple myeloma cells, probably providing autocrine and paracrine proliferation and promoting survival [355].

7.2.4. Extracellular matrix-degrading activities. The first evidence of CD40-medited expression of extracellular matrix-degrading activity referred to the enhanced expression of MMPs in mononuclear phagocytes. Stimulation with a CD154-positive T cells line or via recombinant soluble CD154 induced the expression of interstitial collagenase 1 (MMP-1), gelatinase A (MMP-2; 72-kDa gelatinase), gelatinase B (MMP-9; 92-kDa gelatinase), and stromelysin 1 (MMP-3) mRNA and protein in the human monocytic cell line THP-1 as well as peripheral blood monocytes and monocyte-derived macrophages [142, 143]. MMP gene transcription was induced within 6 h, followed by the respective immunoreactive protein detectable after 12–24 h stimulation. Ligation of CD40 induces the expression of the above-mentioned MMPs as well as interstitial collagenase 3 (MMP-13) and stromelysin-3 (MMP-11) also in human vascular endothelial [356] and smooth muscle cells [179, 180, 357]. These studies also demonstrated that CD40/CD154 interactions do not, or only weakly, affect the expression of endogenous tissue inhibitors of MMPs (TIMPs), suggesting that CD40 ligation shifts the balance toward enhanced enzymatic activity. However, counterregulatory mechanisms for the expression of MMP following CD40 ligation have been reported. In gingival fibroblasts, CD40 signaling resulted in the diminished expression of MMP-1 and MMP-3, while MMP-2 and TIMP-1 production were unaffected [194]. Recent reports showed that CD40 ligation enhanced transcription of the MMP-9 gene in osteosarcoma and Ewing's sarcoma cells [203], further supporting a role for CD40 as a functional receptor engaged in tumor cell survival and malignancy (as also reviewed below). In addition, CD40-mediated expression of matrix-degrading activities has been associated with other chronic inflammatory diseases, such as multiple sclerosis and atherosclerosis [358, 359; see also below].

7.2.5. Procoagulant activities. Studies performed in the early 1980s demonstrated that activated T lymphocytes can induce procoagulant activity on human vascular cells as well as peripheral blood mononuclear cells. Smariga and Maynard [360] demonstrated that platelets stimulate tissue factor expression, the initiator of the extrinsic coagulation pathway, in fibroblasts and smooth muscle cells in vitro. In addition, Gregory and Edgington [361] demonstrated that monocyte tissue factor expression is mediated by CD4 + T helper cells via an apparent contact-dependent mechanism. These findings were later attributed to CD40/CD154-mediated tissue factor expression [143, 362]. Interestingly, the CD40-mediated induction of tissue factor is not inhibitable by IL-10, the cytokine that counteracts various other functions with CD40 [362]. Moreover, the transiently induced tissue factor expression peaks after 6–12 h, differing from other CD154-indued mediators, e.g., cytokines or MMPs. Recently, the CD40-mediated induction of tissue factor on human vascular endothelial cells has drawn attention. Three groups [93, 363, 364], as well as our own [unpublished data], demonstrated that CD40 ligation directly regulates endothelial cell procoagulant activity during inflammatory responses by inducing tissue factor expression and diminishing thrombomodulin expression. CD40 signaling in these experiments was induced via soluble, as well as platelet or T lymphocyte-associated CD154. These findings imply that CD40/CD154 interactions directly modulate endothelial cell procoagulant activity during inflammatory responses, such as wound healing or atherosclerosis.

7.2.6. Other inflammatory mediators. CD40 engagement on human lung fibroblasts increases proinflammatory prostaglandin E_2 synthesis, a process mediated through the induction of cyclooxygenase-2 (Cox-2) [192]. This finding agrees with the report that treatment with anti-CD154 antibodies reduces the oxygen-induced induction of Cox-2 in a mouse model of hyperoxic lung injury [365]. Studies in our own laboratory further demonstrated that ligation of CD40 on atheroma-associated human vascular endothelial and smooth muscle cells also upregulates the expression of Cox-2 [366].

Furthermore, CD40 signaling in macrophages and dendritic cells has been associated with activation of nitric oxide synthase and thus the production of nitric oxide, a molecule that has recently obtained much attention in the context of chronic inflammatory diseases [367–369].

7.3. Apoptosis

Reviewing publications addressing the role of CD40/CD154 in apoptosis fully reveals the controversy of data published on these immune mediators. As discussed above, disparate findings might be explained by the use of (i) cell lines versus primary-culture cells, (ii) different cell types or cell lines, and (iii) similar cell types of different origins or activation status, as reviewed elsewhere [206]. The use of immortalized cell lines for studies of cell death seems questionable and the data obtained with such cell types should be viewed from this perspective. In addition, apoptosis, independent of its triggers, apparently reflects a complex biological process, involving a variety of known (and probably even more unknown) mediators, which act at different levels, and mediate opposing processes, e.g., prevention or induction of apoptosis. CD40-mediated processes in the context of apoptosis have been implicated in both humoral and cellular immunity.

The demonstration that activation through the antigen receptor in combination with a surface glycoprotein recognized by CD40 antibodies prevents centrocytes from undergoing apoptosis probably denotes one of the

first associations of the CD40/CD154 dyad with programmed cell death [370]. Later studies concluded that the protection against Fas-dependent apoptosis afforded by immunoglobulin-receptor engagement may constitute a fail-safe mechanism that eliminates bystander B lymphocytes activated by CD154-bearing T lymphocytes, and at the same time ensures survival of antigen-specific B cells [371]. The rescue of germinal center cells is furthermore thought crucial for the development of high-affinity antibody-producing memory cells. Several studies have demonstrated that ligation of CD40 prevents surface Ig-mediated apoptosis in B lymphocytes [372–375]. These processes correlate with elevated bcl-X, bcl-2 and decreased c-myc expression [376–381], although opposing findings have been reported [382–385]. The controversial findings observed in the regulation of bcl family members are probably explained by their differential regulation during CD40 activation in cells of different location and/or differentiation [386, 387]. Interestingly, bcl-X rather than bcl-2 has been associated with the rescue of centrocytes during germinal center selection [388], and CD40 ligation has been demonstrated to enhance the expression of mediators such as bcl-X_L in several B lymphocyte subpopulations and cell types [387–396].

Although these studies support anti-apoptotic functions of CD40/CD154 interactions, other studies revealed that CD40 ligation induces the expression and/or activation of caspase family members, such as CPP-32 [397, 398], implicating the receptor/ligand dyad in apoptosis. However, whether this is due to a direct mechanism, or whether CD40 ligation primes cells to become more sensitive for apoptosis induced by other pathways (e.g., via CD95) remains unclear. These processes might involve bcl family members and/or the upregulation of Fas [286, 287, 371, 399–402]. As nicely summarized by Kehry [206], directly induced apoptosis has been exclusively observed in cell lines rather than native cells, and thus might be interpreted with the appropriate caution. Accordingly, ligation of CD40 on normal B lymphocytes induces, on B lymphoma cells inhibits proliferation [403].

Furthermore, the nature of an initial activation signal might determine the fate of activated B lymphocytes after (re)engagement of the antigen receptor [404, 405]. Regarding the regulation of B lymphocyte survival, two pathways have been suggested: spontaneous apoptosis occurring in the absence of CD40 ligation during early phases or, following ligation of CD40, germinal B lymphocytes undergo apoptosis when encountering Fas, a process reversible by immunoglobulin receptor signaling. Interestingly, CD40 ligation also sensitizes mature germinal center B lymphocytes to immunoglobulin receptor-mediated cell death [406].

CD40 signaling has furthermore been associated with apoptosis in cells other than B lymphocytes: CD40 ligation assists in clonal expansion of CD4 + thymocytes (but not in activation-induced cell death) [171], rescues monocytes from apoptosis [407], and counteracts Fas-induced apoptosis of human dendritic cells [408], as well as TNF-induced apoptosis in a fibroblast cell line [409]. On the other hand, engagement of CD40 induces apoptosis in neuronal cells [410] and hepatocytes [85], further demonstrating the proapoptotic potential of CD40 signaling.

The finding that CD40 ligation induces death of transformed cells of non-lymphocytic origin, e.g., mesenchymal and epithelial tissue [411], initiated intense research on the importance of CD40/CD154 interactions in the treatment of cancer, e.g., by the induction of apoptosis in carcinoma/lymphoma cells [412]. The demonstration that CD40 ligation also inhibits apoptosis on blood dendritic cells [413] generated further interest in this mechanism for the prevention of drug-induced cell death [414, 415; see also next section]. Furthermore, sensitivity to Fas-induced apoptosis can be increased by CD40 ligation on neoplastic B lymphocytes, indicating an impediment to the development of Fas-based therapies [416]. Accordingly, leukemic B lymphocytes show defective CD40-mediated signal transduction and diminished CD95 receptor expression, which prevents the induction of apoptosis in these cells [417]. However, recent data demonstrating that CD40 ligation directly inhibits human breast carcinoma cells in vitro and in vivo seem to confirm the potential of CD154 for clinical applications [418, 419]. Further studies will have to evaluate whether these effects require interaction with IL-12, as suggested by Esche et al. [420], who demonstrated IL-12-dependent and -independent mechanisms of CD154-induced antitumor immunity [420].

As shown above for other functions, CD154 and cytokines, such as IL-4, IL-10, IL-13, or IFN-γ, also interact during apoptosis [375, 393, 421, 422], as demonstrated for the rescue of human B lymphocytes from cell death by coordinated signaling through CD40 and IL-4 [423].

8. CD40/CD154 in human diseases

In the previous sections, we reviewed the molecular and biochemical characteristics as well as the potential biological functions of the CD40/CD154 dyad. We will now focus on the association of CD40 signaling with human diseases. The majority of studies correlate elevated CD40/CD154 expression with pathological processes, thus suggesting interruption of CD40 signaling as a potential pharmaceutic target. The first association of CD40 with a human disease was related to the discovery of this molecule on human urinary bladder carcinoma and malignant B lymphocytes, e.g., Burkitt

lymphoma cells and Epstein-Barr virus-transformed lymphoblastoid cell lines [2, 3, 99, 120]. These studies, however, lacked evidence of causal association.

8.1. Hyper-IgM syndrome

The first detailed information regarding the importance of CD40 signaling in disease pathogenesis in vivo was provided by the discovery that mutations in the CD154 gene cause an X-linked immunodeficiency, termed the X-linked hyper-IgM syndrome (HIGM) [10, 424, 425]. This primary immunodeficiency disorder is characterized by low levels of IgG, IgA, elevated or normal levels of IgM, and a lack of germinal center formation, thus preventing isotype switching and somatic mutations [16, 156, 424–429]. The decreased levels of the transcript or synthesis of non-functional CD154 result from missense, nonsense, splice site, and/or deletion/insertion mutations [430–433]. A database of CD154 gene mutations causing X-linked HIGM is available [430]. Interestingly, CD4 + T cell lines established from HIGM patients with leaky splice site mutations show an association of full-length and truncated mutants with wild-type CD154. The clinical phenotype of these patients, characterized by a severe impairment of T cell-dependent humoral immune responses, and lack of circulating IgG, IgA, and IgE, as well as memory cells, suggests that this association results in less efficient cross-linking of CD40 [434]. Besides failed expression of CD154 by T lymphocytes, defects in CD40-mediated signal transduction in B lymphocytes cause HIGM [435]. In accordance with these findings, induced genetic deficiency in CD40 and/or CD154 induced similar symptoms in mice [436–438].

8.2. Infectious disease

8.2.1. Viral pathogens. The association between viral infections and the CD40/CD154 receptor/ligand dyad has been studied in detail for the human and simian immunodeficiency virus, demonstrating as early as 1991 that infection of CD4 + T lymphocytes correlates with the expression of CD40 on their cell surface [439]. Although HIV-infected T lymphocytes do not express CD154 [440], and thus cannot account for the hypergammaglobulinemia observed in patients, later studies demonstrated that CD40 signaling might be more than just a bystander in this process, since (i) the interaction of CD4 with gp120 blocks the expression of CD154 on T lymphocytes following anti-CD3-mediated activation (thus probably contributing to the hyporesponsiveness of these cells during HIV infection) [441]; (ii) CD40 signaling affects virus replication, although it is controversial whether it enhances [442–444] or reduces the replication rate [345]; (iii) CD40 ligation induces expression of the chemokines MIP-1α, MIP-1β, RANTES, and MCP-1 [344] and, finally, (iv) the infection of microvascular endothelial cells induced surface expression of CD40 [106]. The latter finding is of particular interest with respect to the observation that HIV infections are associated with the development of aggressive extranodal B cell non-Hodgkin's lymphomas that express CD154 and that interruption of CD40/CD154 interactions inhibits the attachment of those B lymphocytes, potentially reducing HIV-promoted B lymphoma cell growth, which is facilitated by the attachment of lymphoma cells to HIV-infected endothelial cells, probably resulting in malignancies.

Apart from HIV, viral infections in general have been associated with CD40 signaling. Original studies showed a direct anti-viral activity of CD154, which was proposed as a potent effector mechanism of T lymphocytes activated during a virus infection [445]. Infections of CD154-deficient mice with several viruses, including lymphocytic choriomeningitis virus, Pichinde virus, or vesicular stomatitis virus, revealed severely compromised humoral immune responses, suggesting that IgG1 responses are totally dependent on functional CD154, whereas moderate titers of anti-viral IgM and some IgG2a, IgG2b, and IgG3 were produced [446]. Whether effective primary cytotoxic lymphocyte (CTL) responses depend on CD154 is controversial, since opposing findings on the impairment in CTL function and lack of generation of CTL memory cells, germinal centers, and B memory lymphocytes in CD154-deficient mice have been reported [447].

8.2.2. Intra/extracellular microorganisms. Beyond infections with viral pathogens, CD40/CD154 interactions may participate in the immune response initiated by infections via intracellular as well as extracellular microogranisms. Probably due to the X-chromosomal location of the CD154 gene, men rather than women diagnosed for HIGM have an enhanced susceptibility to opportunistic infections, such as *Pneumocystis carinii* pneumonia and *Cryptosporidium* intestinal infections, as discussed in detail elsewhere [28, 448]. Recent studies implicated CD40-mediated IL-12/IFN-γ immune responses as essential to control infections by these intracellular organisms, providing a potential mechanism underlying the susceptibility of patients with HIGM syndrome to infections by opportunistic pathogens [449]. Analysis of the immune response of CD154-deficient mice following infection with the intracellular pathogen *Listeria monocytogenes* and various strains of *Leishmania* [450–453], as well as the extracellular pathogens *Borrelia burgdorferi* [454], *Heligmosomoides polygyrus* [455], and *P. carinii* [456], associated CD40 signaling with disease progression, e.g., via induction of nitric oxide [451] or IL-12 [452, 457, 458]. These findings are in accordance with the report that ligation of CD40 of

peritoneal macrophages prevents infection by *Trypanosoma cruzi* in mice, an effect that requires de novo synthesis of nitric oxide and IL-12 [459]. However, these data probably require further clarification since other studies demonstrated that at least for some intracellular pathogens, e.g., *Mycobacterium* or *Histoplasma capsulatium*, a proper immune response can be achieved independently of CD154 [342, 460].

8.3. Transplantation

Studies in CD40-deficient mice demonstrated that interruption of CD40 signaling reduces the grade of rejection, as originally shown for pancreatic islet allografts [461] and later also for skin and cardiac rejection [462–464], as well as splenocyte [463] or bone marrow [465, 466] transplantation. Interestingly, most previous studies demonstrated only incomplete inhibition of chronic rejection by interruption of CD40 signaling and implicated the requirement for sensitization with further alloantigens, such as CD28 [462, 467] or CTLA-4 [468–471], to achieve long-term survival of the grafts. However, a recent study demonstrated that treatment with anti-CD154 antibodies, without inhibition of further mediators, completely prevented rejection in primates [472]. Thus, a potentially bifunctional role of CD40 signaling might be postulated, mediating processes independent or dependent on other costimulatory pathways, such as the CD28/B7 system, as reviewed elsewhere [473]. Several studies have provided evidence that a reduction in proinflammatory mediators, such as nitric oxide synthase in macrophages and antibody synthesis in B lymphocytes is associated with the benefits observed following interruption of CD40 signaling.

Furthermore, blockade of CD40/CD154 interactions in combination with the inhibition of the CD28/B7 signaling pathways [447] reduced rejection by affecting cytokine production and proliferation of T lymphocytes [462, 463, 466]. With respect to the role of cytokines, the modulation of IFN-γ might be of particular interest, since this cytokine surprisingly facilitates rather than hinders induction of long-term allograft survival, which is considered to be due to limited expansion of activated T lymphocytes [474]. Indeed, IFN-γ-deficient mice demonstrated that CD40 ligation prevents neonatal allotolerance through an IFN-γ- (and IL-12)-dependent pathway [471, 475]. Anti-CD154 antibody-mediated lack of chronic rejection has also been correlated with vascular expression of the 'protective' genes heme oxygenase-1, bcl-x_L, and A20 [476]. Accordingly, expression of CD40 and CD154 is elevated in vivo on infiltrating leukocytes during cardiac and liver allograft rejection, probably interacting with microvascular endothelium [17, 96] and Kupffer cells [477].

8.4. Autoimmune disease

A potential role of CD40/CD154 interactions has been established for several autoimmune disease, including lupus nephritis [478], lupus erythematosus [89, 479], collagen-induced arthritis [480], experimental allergic encephalomyelitis [481–483], and multiple sclerosis [483, 484], as reviewed in detail elsewhere [485]. CD40 signaling has furthermore been associated with early events in the development of spontaneous autoimmune diabetes [486]. Application of anti-CD154 antibodies blocked the development of most of these diseases in the respective animal model [487], probably via modulation of T and/or B lymphocyte responses [488], or Fas-mediated apoptotic processes [489, 490]. Studies in CD154-deficient mice implicated in particular the costimulatory activity of CD154, e.g, via induction of IFN-γ expression in APCs for processes evoking autoimmunity [481, 482]. Among further CD40 signaling-dependent mediators implicated in the progression of autoimmune disease are IL-12 [491, 492], TNF-α [493], and CD80 [463]. Interestingly, T and B lymphocytes of lupus patients [89, 494] express high concentrations of CD154, further supporting the hypothesis of inappropriate CD40 signaling as a crucial participant in the pathogenesis of autoimmune disease, probably by mediating the production of potentially harmful autoantibodies.

In this regard, early studies had revealed that the cell surface of peripheral blood T lymphocytes in rheumatoid arthritis patients differs not only in MHC class II expression, but also in other activation markers, including CD40, from those of healthy persons [129]. Later studies demonstrated elevated expression of both CD40 [104, 495] and CD154 [496, 497] on CD4+/CD8+ T lymphocytes/macrophages of the synovial fluid of rheumatoid patients, where they might modulate development of synovium hyperplasia [188]. Further evidence for the association of CD40/CD154 dysfunction with rheumatoid arthritis has been provided by studies linking the HIGM syndrome with increased incidences of this autoimmune disease [498, 499]. However, the molecular pathways of CD40/CD154 interaction with autoimmune diseases, such as rheumatoid arthritis, remain to be determined.

8.5. Cardiovascular disease

Both immune mediators, CD40 and CD154, are overexpressed in human and experimental atherosclerotic lesions. Functional receptor and ligand are expressed on atheroma-associated endothelial cells, smooth muscle cells, and macrophages in situ and in vitro [18]. Thus, the role of the dyad within atherogenesis has gathered increasing attention during recent years. Among atheroma-associated processes induced via CD40 liga-

tion are the expression of matrix-degrading enzymes, such as MMP-1 (interstitial collagenase type I), MMP-2 (gelatinase A, 72-kDa gelatinase), MMP-3 (stromelysin-1), MMP-9 (gelatinase B, 92-kDa gelatinase), MMP-11 (stromelysin-3), and MMP-13 (interstitial collagenase type III) [142, 143, 179, 180, 356, 357], molecules considered crucial in the processes of plaque progression and finally plaque rupture. The acute thrombotic complications following plaque rupture are mediated through the procoagulant activities of the lipid core, such as tissue factor. Interestingly, CD40 ligation induces tissue factor expression in all three atheroma-associated cell types: endothelial cells, smooth muscle cells, and macrophages [93, 143, 362–364, 500]. Further atheroma-associated processes mediated by CD40 signaling include the induction of proinflammatory cytokines, such as IL-1, IL-6, or IL-8, as well as adhesion molecules, such as ICAM-1, E-selection, or VCAM [20, 22, 24]. Enhanced expression of such molecules, in combination with [probably CD40-mediated; 356] neovessel formation might contribute to the increased extravasation of inflammatory cells and to their accumulation at sites of atherogenesis [501]. This hypothesis is further supported by the finding that CD40 ligation induces the expression of chemokines, a potential pathway by which immune-competent cells might be attracted into deeper layers of the (inflamed) tissue [502]. Another CD40-regulated mediator probably involved in the processes of atherogenesis includes cycloocygenase (Cox)-2 [365]. We recently demonstrated that human atherosclerotic lesions indeed express increased levels of this so-called 'inflammatory' isoform [366], a finding with implications for neoangiogenesis as well [503].

The in vivo relevance of CD40/CD154 signaling in atherogenesis has been demonstrated in animal models. Treatment of hyperlipidemic low density lipoprotein receptor-deficient mice (Ldlr $-/-$) with an anti-murine CD154 antibody significantly reduced the formation of atherosclerotic lesions [175]. The validity of this observation was later confirmed in a study employing CD40L/apoE-deficient mice [504]. Moreover, Aukrust et al. [505] demonstrated enhanced levels of soluble and membrane-bound CD154 in patients with unstable angina. However, whether elevated serum levels of CD154 reflect a causal relationship of this immune mediator with the pathogenic processes of atherogenesis or whether this inflammatory marker is just a bystander remains to be determined.

Although the mechanisms of CD40 and CD154 expression within the atherosclerotic lesion are only poorly understood, one can reasonably postulate involvement of 'classical' activators such as IL-1, TNF-α, and in particular IFN-γ, which enhance both CD40 and CD154 expression in vitro and which are expressed within the lesion [506]. In addition, a recent report demonstrates that lysophosphatidylcholine, which accumulates within atherosclerotic plaques, also enhances CD154 expression at least in CD4+ T lymphocytes [507].

8.6. Cancer

Cancer was probably the first human disease associated with the CD40/CD154 signaling pathway, as CD40 was first described on the surface of neoplastic B lymphocytes and malignant cell types of non-hematopoietic origin [2, 3, 349, 99, 120]. Later studies extended these findings, associating CD40/CD154 with epithelial neoplasia [42, 99] as well as chronic lymphocytic, acute lymphoblastic, and hairy cell leukemia, non-Hodgkin's lymphomas, nasopharyngeal carcinoma, osteosarcoma and Ewing's sarcoma, myeloma, Hodgkin's disease, melanoma, and bladder transitional-cell carcinomas [115, 123, 166, 169, 197, 198, 203, 508–511]. In accordance with the neoplastic nature of these CD40 sources are studies demonstrating that ligation of CD40 suppresses apoptosis in various cell types [384, 512]. As outlined above, CD40 ligation induces anti-apoptotic processes and stimulates DNA synthesis, triggering activated B lymphocytes to enter S phase, and eventually induces proliferation in those cells [120, 299, 513, 514]. Moreover, CD40 ligation rescues malignant and normal B lymphocytes from induced growth arrest [515]. The finding that ligation of CD40 enhances DNA synthesis and prevents apoptosis suggests a potential role for the CD40/CD154 signaling dyad in the initiation and/or evolution of cancer cells. Knowledge regarding the molecular mechanisms underlying these pro- and anti-apoptotic functions, however, remains sparse. In particular, the interaction of the CD40/CD154 dyad with the Fas signaling pathway has been implicated in malignancy. Cell type as well as timely sequence of interactions between the two mediators probably play a crucial role in the fate of carcinoma cells. Ligation of CD40 before that of CD95 renders Hodgkin's disease cell lines refractory to Fas-induced apoptosis [516]. This hypothesis is supported by recent studies revealing that expression of CD40 by B lymphocytes from chronic lymphocytic leukemia patients is similar to that of normal patients', whereas CD40 signaling is defective. This correlates with diminished CD95 expression and lack of induction of apoptosis following ligation of CD95 in these cells, demonstrating a potential pathway of long-term survival in leukemic cells [417].

In addition to B lymphocytes, CD40 signaling on other cell types, such as endothelial cells in Karposi's sarcoma [174], benign juvenile and lobular capillary hemangiomas [172], epidermal tumors [185], and renal cell carcinoma [173] is also considered relevant for the progression of the respective disease. Moreover, T lymphocytes have been implicated in the finding that leukemia cell-mediated downmodulation of CD154 on activated T lymphocytes accounts for some of the acquired immune defects of patients with chronic lymphocytic leukemia [517].

In accordance with the elevated expression and potential functions of CD40 in cancer, the immune mediator may provide a marker for tumor cells [198] as well as a potential target for therapeutic intervention in the treatment of B cell lymphomas and other forms of cancer [202]. Original studies revealed that treatment with anti-CD40 antibodies inhibits the proliferation of murine B lymphoma cells [403, 518] and human breast carcinoma cells in vitro and in vivo [204]. Treatment with anti-CD40 antibodies also diminishes the development of Epstein-Barr virus-mediated human B cell lymphoma [519]. The potential role of CD40/CD154 interaction in cellular tumor immunity in vivo has been further established in CD154-deficient mice, which lack the protective immunity against syngeneic tumors generated in wild-type mice by the administration of tumor vaccines [520, 521]. Moreover, ligation of CD40 eradicates syngeneic lymphoma in mice and provides protection against repeated tumor challenge, which French et al. [522] interpret as a potential immunization against syngeneic tumors.

Similar studies have revealed that transducing tumor cells with CD154 can mediate rejection and/or create a long-lasting systemic immune response capable of impeding growth of unmodified neuroblastoma cells [523, 524], and that CD154 can enhance the immune response against leukemia [525] and squamous cell carcinoma [526]. Thus, patients with chronic lymphocytic leukemia, who demonstrate lack of CD154 on peripheral blood T lymphocytes, acquire an immunodeficiency with characteristics similar to those of patients with inherited defects in the gene encoding CD154 [517]. Other studies, however, demonstrated that serum of patients with chronic lymphocytic leukemia contained elevated levels of biologically active soluble CD154, and that the ligand prolongs survival of leukemia cells and mediates their resistance to apoptosis, at least in vitro [88, 383]. These findings imply that both interruption or enhancement of CD40/CD154 interactions might benefit patients, depending on the particular nature of the cancer treated. In accordance with this hypothesis, experiments employing BALB/c mice demonstrated that challenge with a lethal dose of tumor can be overcome by vaccination with plasmids encoding CD154, preventing development of metastatic tumors, and implicating CD154 cDNA treatment as a potential vaccine adjuvant [458]. Results of another study support the concept that transduction of tumor cells with recombinant CD154 adenovirus vector might provide a treatment for cancer immunotherapy, since treatment of established subcutaneous tumors resulted in sustained tumor regression and tumor-free status in $>60\%$ of the mice [527]. Furthermore, recent studies suggest that CD154 gene transfer into tumor cells may induce tumoricidal activity of host alveolar macrophages, thus further supporting the potential for this strategy of cancer gene therapy [528]. However, there are potential limitations to this therapeutic approach. Injection of a retroviral vector containing the cDNA for murine CD154 in CD154-deficient mice effectively stimulates humoral and cellular immune functions short term, but causes T cell lymphoproliferative disorders long term [529]. Furthermore, activation of CD40/CD154 signaling might prove counterproductive in some circumstances, since ligation of CD40 inhibits the cytotoxic effect of the anti-cancer drug doxorubicin in non-Hodgkin's lymphoma cell lines, probably by abrogating caspase-3 activation, thus mediating resistance to chemotherapy in lymphoma cells [415]. An alternative strategy focuses on the use of immunotoxins. The demonstration that immunotoxins targeted against CD40, either in chemical conjugate form [115] or as single-chain immunotoxins [530, 531], mediate anti-tumor activity in vitro and in vivo suggested the potential for therapeutic use, which will require further confirmation.

8.7. Other human diseases

CD40/CD154 interactions have recently been implicated in lung disorders, although expression of the molecules on basophils [134] within lung tissue was described nearly a decade ago. Later studies localized CD40 also on fibroblasts [186], macrophages [532], and endothelial cells [24] of lung tissue, as well as bronchial epithelial cells [98]. CD40 was initially associated with lung disorders by the finding that ligation of CD40 induces expression of IgE as well as inflammatory mediators, such as cytokines or Cox-2 [192, 365, 533] in human lung mast cells, blood basophils, and eosinophils implicating a role for the CD40/CD154 dyad during allergy [79]. Accordingly, treatment of wild-type mice with CD154 protein induced pulmonary inflammatory reactions, which were not observed in identically treated CD40 gene-deficient mice [534]. Furthermore, artificially induced airway inflammation was not attenuated in CD40-deficient mice compared to wild-type mice [533, 535]. Recent studies provide evidence that bone marrow-derived CD40+ cells are required for the induction of pulmonary inflammation and that alveolar macrophages, B and T lymphocytes rather than fibroblasts, epithelial cells, or endothelial cells can establish pulmonary inflammation [536]. Interruption of CD40/CD154 interactions has recently been suggested as a potential pharmaceutical target for intervention for oxygen-induced acute respiratory distress syndrome [365], and radiation-induced pulmonary toxicity [537].

In summary, elevated expression and interaction of the CD40/CD154 receptor/ligand dyad is associated with the progression of many prevalent human diseases. Accordingly, animal models have revealed evidence for the

therapeutic potential of anti-CD40L antibody treatment However, whether these techniques can be applied to humans remains to be determined, since treatment with ('humanized') antibodies has obvious limitations. Unfortunately, no effective synthetic small molecule inhibitors of CD40/CD154 interactions have been reported, leaving humanized antibodies as the most propagated choice for therapeutic intervention.

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