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Graft 2001; 4; 188

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CD40 is a cell surface receptor which belongs to the TNF-R family. It was first identified and functionally characterized on B lymphocytes. The ligand for CD40 (CD40-L/CD154) is expressed mainly on activated T cells and plays a pivotal role in T cell-dependent B cell responses. However, in recent years it has become clear that CD40 has a much broader expression pattern, including expression on monocytes, dendritic cells, endothelial cells and epithelial cells. Therefore it is now thought that CD40 plays a more general role in immune regulation. The present paper reviews recent developments in this field of research with an emphasis on its expression and function on cells other than B lymphocytes (see Biotoon pp. 220-221), on CD40 signal transduction and on the function of CD40-CD40L in transplant rejection.

ABBREVIATIONS:

TRAF	TNF receptor associated factor
Jak3	Janus protein tyrosine kinases
ERK	p44 MAP kinase
p38	p38 MAP kinase
JNK	c-Jun NH2-terminal kinase
STAT	signal transducers and activators of transcription
NFκB	nuclear factor kappa B
NFAT	nuclear factor of activated T cells

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Expression and Function of CD40 on Hematopoietic Cells

B Lymphocytes. Extensive studies on CD40 activation of B cells in vitro, have demonstrated that CD40 activation has major effects on many steps of the B cell natural history.[1-3] CD40 ligation activates resting B cells as shown by their increase in size and expression of new surface molecules involved in homotypic and heterotypic aggregation (CD23, VLA-4), T cell costimulation (CD80/CD86) and increased expression of MHC class I and II molecules and the TAP transporter. Furthermore, CD40-activated B cells secrete a myriad of cytokines which may act as autocrine and paracrine growth as well as differentiation factors. CD40 acts in concert with either cytokines or other receptor-ligand interactions for most biological functions of B cells, including the generation of germinal centers, isotype switching, induction of somatic mutations and selection of high affinity antibodies.

Monocytes and Dendritic Cells. Primary human monocytes either freshly isolated or cultured for 48 h show low but detectable CD40 surface protein expression.[4] Strong expression is observed on cultured plastic adherent human monocytes.[5] Monocytes stimulated by CD40L transfected cells secrete low amounts of IL-6 and IL-8 and turn on their tumoricidal activity against a melanoma cell line.[4,6] Finally, CD40 ligation activates NO synthesis as shown by the inhibition of T-cell-induced nitric oxide production by mouse macrophages using anti-CD40 antibodies.[7]

The expression of CD40 on antigen presenting cells like monocytes and dendritic cells has an important role in vivo.[8,9] Activation of the CD40 receptors is one of the critical signals which allow the full maturation of dendritic cells into the most powerful antigen presenting cells.[10] CD40 ligation on dendritic cells results in:

- increased expression of costimulatory molecules such as CD54/ICAM-1, CD58/LFA-3, CD80/B7-1, CD86/B7-2;
- the secretion of cytokines (such as IL1, IL6, IL8, IL10, IL12, TNF-α, MIP1α) and enzymes such as matrix metalloproteinase (MMP);
- an enhanced survival of these cells
- NO synthesis;
- promote differentiation of DC from $CD34^+$ hematopoietic progenitors.[11-14]

Therefore the interaction between CD40 and CD40L has important consequences for both APC function and T cell function. The full impact of

CD40 for DC biology is outside the scope of this paper and is reviewed elsewhere.[15,16]

T Lymphocytes. CD40 expression has been observed on rare $CD4^+$ and $CD8^+$ T cell clones,[17] as well as on T cells isolated from patients with rheumatoid arthritis.[18] Human resting T lymphocytes activated by a soluble trimeric CD40L or by CD40L transfected cells express activation molecules such as CD25, CD69 and CD40L.[19] Both αβ and γδ mouse T cells are stimulated in a similar way, resulting in enhanced proliferation, induction of activation markers (CD25, CD69) and secretion of cytokines (IL-2, IFN-γ and TNF-α).[20,21]

CD40 on Non-Hematopoietic Cells

Apart from hematopoietic cells, the expression of CD40 has now been observed on many other cell lineages. Although the expression is generally low in normal tissues, the molecule is clearly upregulated under various pathological conditions. In accordance, expression can be observed on many cultured cell types including endothelial cells, epithelial cells and fibroblasts.

Endothelial Cells. Endothelial cells of microvessels in the skin express CD40 intensely.[22,23] In agreement, cultured human umbilical vein endothelial cells (HUVEC) constitutively express the CD40 antigen. The expression of CD40 is significantly upregulated by IL-1α, TNF-α, IFN-γ and IFN-β, but not by IL-4.[23] Crosslinking of CD40 on cultured HUVEC results in a marked upregulation of ICAM-1 (CD54), VCAM-1 (CD106) and E-selectin (CD62E), thus resulting in an increased ability to bind leukocytes.[22,23] Activation of endothelial cells also results in increased production of inflammatory mediators.[24,25] Interaction between endothelial cells and T cells might also result in a positive feedback for CD40L expression via CD2/LFA3 expression.[26]

Interestingly, CD40 expression on endothelial and other vascular wall cells such as smooth muscle cells is important for the development of atherosclerosis.[27] In vitro activation of these cells via CD40 results in expression of tissue factor,[28] interleukin-1 converting enzyme (ICE; caspase-1)[29] and metalloproteinases, including stromelysin-3.[30,31] An important remaining question is the source of CD40L in these vascular lesions, which might include activated T cells, vascular cells themselves,[32] or maybe more likely, activated platelets.[33]

Epithelial Cells. The CD40 antigen was identified initially with the mAb S2C6 which was generated from mice immunized with a urinary bladder carcinoma.[34] Subsequently, the CD40 antigen has been identified on carcinomas of other origin such as colon, prostate, breast and lung, as well as on melanomas. Interestingly, on carcinomas it has been demonstrated that CD40 activation results in induction of cell death.[35-37] Similarly CD40 ligation appears to inhibit the proliferation of diffuse B cell lymphomas.[38,39] and could therefore provide a new therapeutic tool in some specific tumor cases.

CD40 is also expressed on non-malignant epithelial cells, including the CD45-negative stromal cell population in cortex and medulla of the human thymus.[40] IL-1α, TNF-α and IFN-γ significantly upregulate CD40 levels on cultured epithelial cells, and these cells are induced to secrete GM-CSF upon CD40 ligation.[40] CD40 expression on thymic epithelial cells might also provide a costimulatory signal for human thymocytes.[41]

In renal interstitial inflammation, as during allograft rejection, tubular epithelial cells (TEC) play an active role in the production of pro-inflammatory mediators and a wide variety of chemokines.[42] In normal tissue, only low expression of CD40 is observed on tubules, but it is strongly increased under inflammatory conditions. In vitro, tubular CD40 activation enhances the production of chemokines like IL-8, MCP-1 and RANTES.[43] More importantly, simultaneous addition of pro-inflammatory cytokines like IL-1 or IL-17 showed strong synergistic effects on the production of these mediators.[44,45] Moreover, it was demonstrated that the cytokines IL-4 and IL-13 specifically enhanced CD40-induced RANTES production,[46] whereas they did not affect the production of other chemokines. These data suggest a complex network of interactions between epithelial cells and activated T cells, as also shown by in vitro cocultures.[47]

Networks of interaction and crosstalk between activated T cells and epithelial cells via CD40-CD40L is most likely not restricted to the kidney. For instance, CD40 is also expressed on skin keratinocytes and crosslinking of the receptor results in further differentiation of these cells and an increased IL-6 production.[48-50]

Fibroblasts and Other Non-Hematopoietic Cells. Tissue fibrosis is characterized by the local presence of infiltrating mononuclear cells and activation of

DENDRITIC CELLS:

Antigen-presenting cells which play a critical role in the induction of immune responses, but are also instrumental for the induction of tolerance. Subsets of DC have been identified, but the exact relations between functions, lineage, origin and activation states are still a matter of debate.

TUBULAR EPITHELIAL CELLS:

Epithelial cells in the kidney which not only play a critical role in water and salt homeostasis, but are also an active participant in local inflammatory responses.

fibroblasts.[51] CD40 is expressed on various fibroblast populations, including cells derived from skin, lung, kidney and synovium. Ligation of CD40 on synovial fibroblasts results in an enhanced growth, expression of adhesion molecules and production of various cytokines, including IL-6, GM-CSF and VEGF.[52-54] Lung fibroblasts start to express higher levels of cyclooxygenase-2 upon CD40 activation.[55] It has been suggested that expression of CD40 on fibroblasts from different organs might associate with different chemokine expression profiles.[56] Therefore, cells of different origin should be studied separately, since they might have different biological potential.

CD40 is expressed on follicular dendritic cells (FDC) of secondary lymphoid organs as shown by immunohistology and also on freshly isolated FDC. Interaction between activated T cells and an FDC-like cell line results in enhanced growth and increased expression of ICAM-1, mediated via CD40-CD40L interaction.[57]

Reed Sternberg (RS) cells, characteristic of Hodgkin's lymphoma, display enhanced clonogenic capacity and colony cell survival after engagement of CD40 by soluble CD40L.[58] Crosslinking CD40 results in enhanced production of IL-6, IL-8 TNF-α and LTα, as well as upregulation of ICAM-1 and CD80.[59]

Finally, there are reports on CD40 expression on human muscle cells, regulating the production of various cytokines,[60] whereas crosslinking of CD40 expressed on human hepatocytes results in the induction of apoptosis via the Fas-FasL pathway.[61] Understanding the different biological functions of CD40 on different cell types will depend on the insight in mechanisms of CD40 signal transduction.

CD40 Signal Transduction

Coupling of CD40 to different signaling pathways has been better understood by the identification of a family of associated proteins: TRAF (TNFR associated factor)[62] At least four TRAF molecules have been demonstrated to associate with the CD40 cytoplasmic tail (TRAF2, TRAF3, TRAF5, TRAF6). Deletion mutants of the intracellular region of CD40 showed association of TRAF2, TRAF3 and TRAF5 to one region (residue 246-269), whereas TRAF6 associated with a separate domain (residue 230-245)[63] In accordance, pepscan analysis of CD40 identified PVQET (residue 250-254) as the minimal unit for TRAF1, TRAF2 and TRAF3 binding, and QEPQEINF (residue 231-238) as the minimum for TRAF6 binding (Fig. 1).[64,65] To unravel this complex network and to understand the in vivo role of the different TRAF molecules, several members have been genetically inactivated in mice.

Genetic inactivation of TRAF3, TRAF2 and TRAF5 respectively, revealed no gross abnormalities at birth, but the mice died early in life.[66-68] Functional analysis of signaling pathways showed alterations in NF-κB and JNK activation and the expression of several activation markers, but suggested a certain level of redundancy. Generation of TRAF6 KO mice showed low birth rates of -/- mice, severe osteopetrosis and early death.[69,70] TRAF6 is also critical in the signal transduction induced by the Toll/IL1-R family.[71-74] Accordingly, the absence of TRAF6 not only interferes with CD40-mediated B cell proliferation, but also B cell proliferation induced by LPS or induction of iNOS in macrophages by IL-1 or LPS.[69,70]

Recent studies identified a domain (pre-ligand assembly domain; PLAD) in the extracellular part of CD40, which is responsible for the assembly of a trimeric receptor complex in the absence of a ligand.[75] It remains to be determined whether there is a signaling function for ligand-independent trimers and what is the exact consequence of ligand binding. Upon activation, CD40 together with associated TRAF molecules move to lipid microdomains which brings them in close vicinity of different src kinases.[76,77] A wide variety of kinases have been implicated to become activated by the CD40 pathway, including PTKs, PKA, PI3K, JAK3, NIK, JNK, ERK and p38 MAPK. However, it remains to be established which pathways are important under certain conditions or in specific cell types. For instance, although the NF-κB inducing kinase (NIK) was shown to be essential for B-cell activation, it seems to be dispensable for activation of dendritic cells.[78] Similarly, TRAF6 seems to be an intermediate in CD40-induced NF-κB activation in 293 epithelial cells, but not in B cells.[79]

CD40 and Transplantation

Interference with CD40 activation prolongs the survival of experimentally transplanted organs. These models have been mostly limited to murine models, including heart, skin, aorta and pancreatic islets allografts, as well as the respective xenografts.[80-83] The contribution of CD40-

TRAF:

A group of intracellular proteins, TNF-receptor associated factors, which on the one hand associate with the intracellular parts of receptors of the TNFR family, including CD40, and on the other hand connect to different signal transduction pathways.

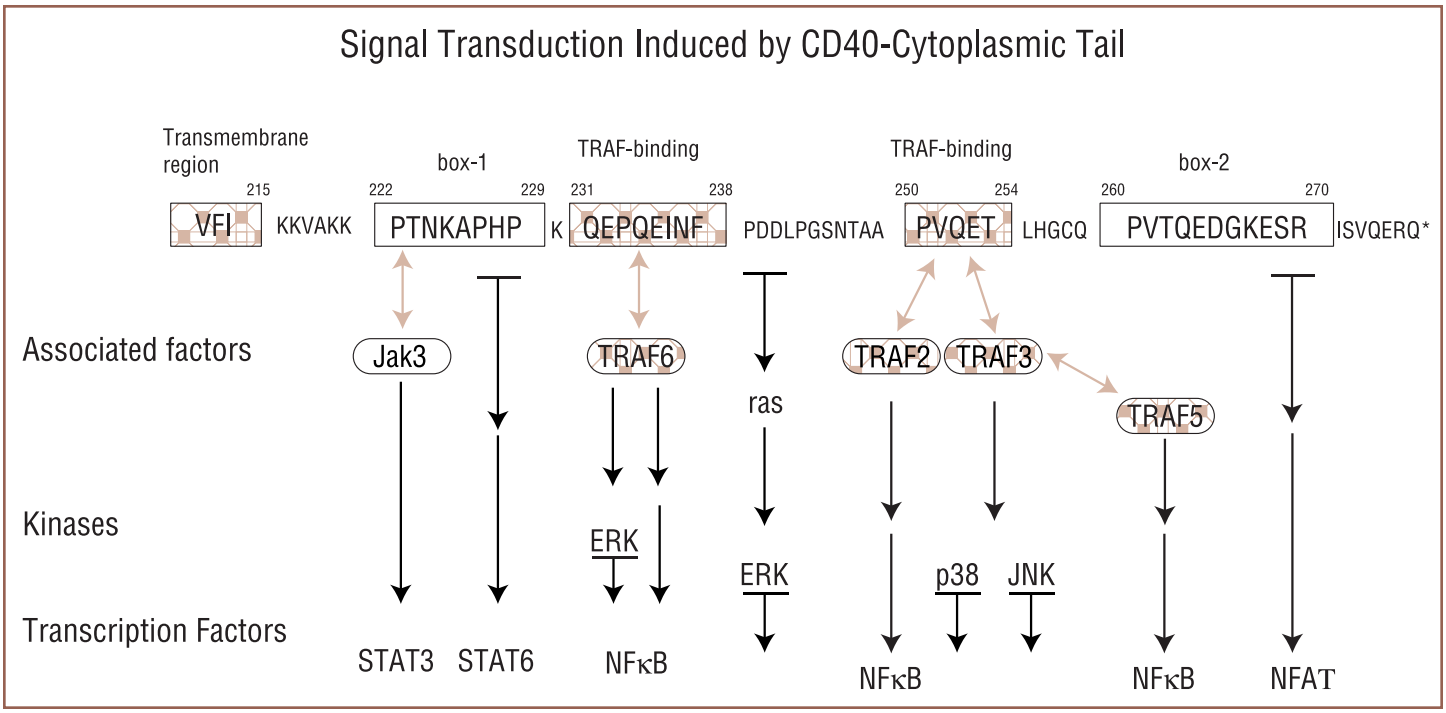

Figure 1. Schematic representation of the amino acid sequence of the intracellular region of human CD40 (single letter code and numbering of mature protein). Several domains have been identified which either directly associate with identified adaptor molecules such as TRAFs or which have been implicated in the induction of specific pathways, ultimately leading to gene activation.

CHEMOKINES:

A group of small polypeptides which are responsible for the chemoattraction of leukocytes to sites of inflammation, or in the normal trafficking of these cells. Specific interaction with chemokine receptors, which are differentially expressed on different cell types, will determine the composition of cellular infiltrate.

HUMANIZED ANTI-CD40L:

A monoclonal antibody specific for CD40L. which was initially raised in mice, but with all constant regions of the Ig molecule replaced for the human sequence. Only the antigen recognition site is still of mouse origin which should diminish the in vivo anti-mouse response observed with classical mouse monoclonal antibodies.

CD40L interactions in the process of T cell priming, differentiation and effector functions has been extensively reviewed.[9,84-86] However, it has been shown that the results in transplantation models are strongly dependent on the strain combinations used. In several cases, rejection seems to occur independent of CD40-CD40L and is mediated by CD8 positive T cells.[87,88] Moreover, it has been shown that the Th1 or Th2 background of the mice affects the result of anti-CD40L blockade.[89]

Treatment regimens have been different in the various studies, ranging from once at the time of transplantation to repeated administration every 72 hours to prevent chronic rejection in aortic allografts.[90] Local expression of CD40L has been found in human renal and heart allografts, and its expression seems to correlate with rejection.[45,91-93]

Studies with a humanized anti-CD40L antibody have been performed in renal allotransplantation in rhesus monkeys. An extended course of anti-CD40L as the only treatment over a 6 months period resulted in long-term survival.[94,95] Importantly, the organs were not rejected when treatment was stopped, and graft survival in this group reached up to 10 months post-treatment. Combinations of current immunosuppressive drugs, including steroids, MMF, and FK506, seem to counteract the beneficial effect of anti-CD40L. The same humanized Ab has been used in a primate model of cardiac transplantation, showing improved survival and less vascular pathology.[96]

Conclusion

Research on CD40 has over the years shifted from a restricted role in the regulation of humoral immunity to a much broader role in immune activation and inflammation. Although the present data do not provide sufficient evidence to show permanent transplant tolerance, the data are promising and have resulted in a phase II human clinical trial in renal allograft recipients. Despite discontinuation of this trial due to thrombo-embolic complications,[97] it is expected to be resumed. In view of the broad expression of the CD40 receptor on many cells with a potential role in allograft rejection, further detailed analysis is needed to unravel the mechanism of action of CD40L blockade.

Acknowledgments. The research of C van Kooten has been made possible by a fellowships of the Royal Netherlands Academy of Arts and Sciences (KNAW). Part of the original work presented in this review was performed within the framework of the EU concerted action BMH4-CT98-3631 and supported by the Dutch Kidney Foundation (NSN C98.1749)

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