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Special Article

The Biology of the Human Ligand for CD40

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INTRODUCTION

CD40 refers to a 50-kD glycoprotein which has been reported to be expressed on the surface of a variety of cell types including B lymphocytes, follicular dendritic cells, thymic epithelium, and some epithelial carcinomas (1). It is a member of the tumor necrosis factor (TNF) receptor family of molecules, which includes both forms of TNFR, the nerve growth factor receptor, CD30, and the recently described Fas antigen (1–4). Studies using monoclonal antibodies (mAb) to CD40 have indicated a diverse array of biological activities occurs as a result of signaling through CD40. These include proliferation of anti-IgM cross-linked B cells (5, 6), secretion of IgE, IgG, or IgM in the presence of various cytokines (7–12), and the rescue of germinal center centrocytes from apoptosis (13–15). In addition, Galy and Spits have shown that triggering CD40 on thymic epithelial cells in the presence of gamma-interferon (γIFN) and interleukin-1 (IL-1) results in GM-CSF release (16), while other groups have shown that both homotypic and heterotypic cell adhesion can be induced by mAb to CD40 (17, 18). Functional data such as these led to the search for, and the identification of, a naturally occurring ligand for CD40.

The human ligand for CD40 (CD40L) is a 33-kD type II membrane glycoprotein expressed primarily, but not exclusively, on the surface of activated T helper cells (19–22). Not surprisingly, it exhibits significant amino acid homology to TNF, particularly in the TNF binding domain (23). Recombinant CD40L has been shown to stimulate the proliferation of B cells in the absence of a costimulus and, to induce immunoglobulin secretion in the presence of an appropriate cytokine (19).

Unexpectedly, restriction fragment length polymorphism (RFLP) analysis in the mouse demonstrated X-chromosomal inheritance. *In situ* hybridization and hamster/human hybrid mapping localized the gene for the CD40L to the q26 region of the human X chromosome (20, 24, 25). Subsequent studies performed by a number of groups identified CD40L gene defects as the causative factor in a severe immune deficiency known as X-linked hyper-IgM (HIGM), a syndrome typified by elevated levels of serum IgM and low or nonexistent levels of IgG, IgA, and IgE (24–28). To date, 12 CD40L mutations have been identified from HIGM patients, 11 of which occur in the extracellular portion of the molecule. Correlation of the CD40L mutations in HIGM patients with their distinctive clinical phenotype has provided new insight into the biology of the CD40L. The observation that CD40L acts in the stimulation of monocytes, and costimulation of T cells, suggests an explanation for the common complication of neu-

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tropenia and other severe immune defects associated with X-linked HIGM.

IDENTIFICATION OF THE CD40L

Antigen-specific activation of B cells is considered a two-step process. In the initial inductive phase, resting T cells come in contact with B cells which display antigen in the context of class II MHC on their cell surface. In the second or effector phase, the T cells involved in this cognate interaction also become activated and, through the secretion of cytokines and expression of activation induced cell surface molecules, drive the differentiation of B cells into immunoglobulin secreting plasma cells. Strong evidence for an activated T helper cell surface molecule which could provide B cell help came from studies using fixed, activated T cells (29) or T cell membrane preparations (30–34). Because of these data, a murine thymoma cell line, EL-4, was selected as a potential source for the CD40L. Using a CD40–human IgG1 chimeric fusion protein (35) and an unusually sensitive method of expression cloning (36) a cDNA encoding a murine CD40L was identified (22). Nucleic acid cross-hybridization techniques allowed the identification of a human homologue, and shortly after publication of the murine CD40L sequence, several groups described the human CD40L, which is 78% identical to murine CD40L at the amino acid level (Genbank accession number X67878) (19–21). The human CD40L is a 261-amino acid, 33-kD, type II integral membrane protein comprised of a 22-amino acid cytoplasmic domain, a 24-amino acid transmembrane domain, and a 215-amino acid extracellular domain. It contains a single N-linked glycosylation site, which is likely utilized. Northern blot analysis indicates two forms of CD40L mRNA are expressed in activated T cell lines, a larger, ~2000-bp species and a slightly smaller species (19, 20). Hybridization with oligonucleotides derived from different portions of the CD40L coding region indicate that the smaller mRNA does not encode a soluble form of CD40L (19). Thus far, there is no evidence at either the protein or the RNA level that this CD40L protein exists in a naturally occurring soluble form.

THE BIOLOGY OF THE CD40L

Expression of the CD40L at the level of RNA appears restricted to activated T cells (19–21) and some stromal cell lines (unpublished observations), although an exhaustive analysis has not yet been performed. Two-color flow cytometry studies indicate that CD40L expression is confined largely to the $CD4^+$ subpopulation, although some $CD8^+$ cells also appear positive (19). The role of CD40L on these $CD8^+$ cells is not clear.

Initial studies in our lab on the biological activities of recombinant CD40L were performed using transfected CV-1/EBNA cells (a monkey kidney cell line), which express high levels of CD40L (19). This membrane-bound CD40L is directly mitogenic for B cells derived from blood or tonsils in the absence of any costimuli. Inhibition assays using CD40.Fc or blocking antibody to CD40 suggest that the mitogenic activity appeared due to CD40L expression alone, and not CD40L in the context of an endogenous cell surface molecule. The CD40L-induced proliferation of tonsil B cells was significantly enhanced in the presence of IL-2, IL-4, or IL-10 (37) and could be inhibited by the addition of tumor growth factor-beta (TGFβ). IL-5, TNFα, and γIFN do not appear to augment CD40L-induced proliferation when added independently to cultures. However, the addition of any of these three cytokines in the presence of IL-4 results in some enhancement of proliferation. The IL-4 and IL-10 augmentation of CD40L-induced proliferation is similar to results which were described previously by Banchereau and his colleagues using a culture system containing immobilized CD40 mAb (6, 8, 11). Interestingly, the costimulatory effects of IL-2 and IL-5 appear evident only when signaling through CD40 occurs as a result of CD40L interaction (37).

The CD40L induces immunoglobulin secretion only in the presence of appropriate cytokines. The choice of cytokine is critical to the production of a particular isotype. In the human system, the addition of IL-4 results in IgE and IgG4 secretion. Either IL-2 or IL-10 functions as a potent costimulus for the production of IgM, IgG1, IgG2, IgG3, or IgA (38). Other experiments have shown that the secretion of all isotypes derived from our CD40L containing *in vitro* culture system is inhibited by TGFβ (37).

Recent work in our lab and others has involved the use of soluble forms of the CD40L. Based on amino acid homology between CD40L and other members of the TNF family (Table I) (23), the native form of CD40L likely exists in a trimeric or oligomeric state (39). Consistent with this, simple monomeric forms of recombinant CD40L bind

Table I. Percentage Amino Acid Identity Between the TNF Binding Domains of Various TNF Family Members

	HuTNFα	MuTNFα	HuTNFβ	MuTNFβ	HuCD40L	MuCD40L	HuCD27L	HuCD30L	MuCD30L
HuTNFα	100	80	36	39	27	25	18	16	18
MuTNFα		100	37	41	25	24	21	15	18
HuTNFβ			100	79	24	22	18	14	18
MuTNFβ				100	25	25	18	17	18
HuCD40L					100	75	22	12	17
MuCD40L						100	18	15	16
HuCD27L							100	12	13
HuCD30L								100	78
MuCD30L									100

CD40 but do not signal effectively (unpublished observations). Alternative chimeric proteins have been created using the extracellular portion of the murine CD8 protein fused to the extracellular domain of the CD40L. The CD8 protein portion drives the formation of dimers and oligomers when this fusion protein is expressed *in vitro.* This protein functions to drive B cell proliferation but requires a costimulus such as PMA or CD20 mAb (21). We have created two forms of chimeric CD40L: a human (hu) IgG1–CD40L protein which dimerizes as a result of the disulfide bonds in the hinge region of the Ig constant domain and a trimeric form, which is composed of a 30-amino acid leucine zipper motif upstream of the extracellular domain of the CD40L (40). The CD40L.Fc is directly mitogenic on resting B cells. In contrast, B cells cultured with the trimeric CD40L exhibit low-level proliferation in the absence of costimulus but perform at a level comparable to the membrane-bound CD40L in the presence of anti-IgM. In culture with the appropriate cytokine, both forms are capable of inducing immunoglobulin secretion. These data strongly suggest that cross-linking of CD40 is important for signaling through the molecule.

Flow cytometric analysis of CD40L activated B cells has shown that several antigens are induced as a result of CD40 signaling. These molecules are induced in the absence of costimuli and include CD23 and B7 (unpublished observations).

DEFECTS IN THE CD40L GENE ARE RESPONSIBLE FOR X-LINKED HYPER-IgM IMMUNODEFICIENCY SYNDROME

The mouse chromosomal location of the *CD40l* locus was determined by interspecific backcross analysis. Southern blotting was used to identify informative restriction fragment length polymorphisms (24, 41). The *CD40l* locus mapped to the X chromosome approximately 1.5 cM distal of the *hprt* gene. This suggested that the human CD40L gene might also be linked to the HPRT locus, thereby mapping to Xq26. This was confirmed using *in situ* hybridization in our laboratory (24) and that of Aruffo and colleagues (25) and by Graf *et al.* (20).

The potency of recombinant CD40L protein in *in vitro* assays, together with its provocative chromosomal location, suggested that defects in the CD40L gene might be responsible for a known immunodeficiency. At the time, there were at least five described X-linked immunodeficiencies for which the underlying defect was unknown. These included severe combined immunodeficiency (XSCID), agammaglobulinemia, Wiskott–Aldrich syndrome, X-linked lymphoproliferative syndrome (XLP), and X-linked hyper-IgM (HIGM). Of these, XLP, X-linked HIGM, Wiskott–Aldrich, and XSCID were syndromes which seemed likely to involve a defect in T cell function. Also, X-linked HIGM was reported to map to the region of Xq24–Xq27 (42, 43). These data made the X-linked HIGM syndrome a strong candidate for a disorder associated with defects in the CD40L gene. PCR analysis of cDNA derived from patient PBL mRNA quickly showed that the X-linked HIGM patients, but not other X-linked immunodeficiency patients, exhibited point mutations in the extracellular domain of the CD40L.

X-linked HIGM syndrome is a rare disorder characterized by normal to elevated levels of IgM and decreased or absent serum levels of IgG, IgA, and IgE (44, 45). Diagnosis is frequently made following recurrent bacterial infections in the first few years of life (44). Intravenous administration of γ-globulin and aggressive use of antibiotics are used, but there is a general consensus that the functional immune deficiency is relatively severe. Interestingly, affected males exhibit an unusually high incidence of

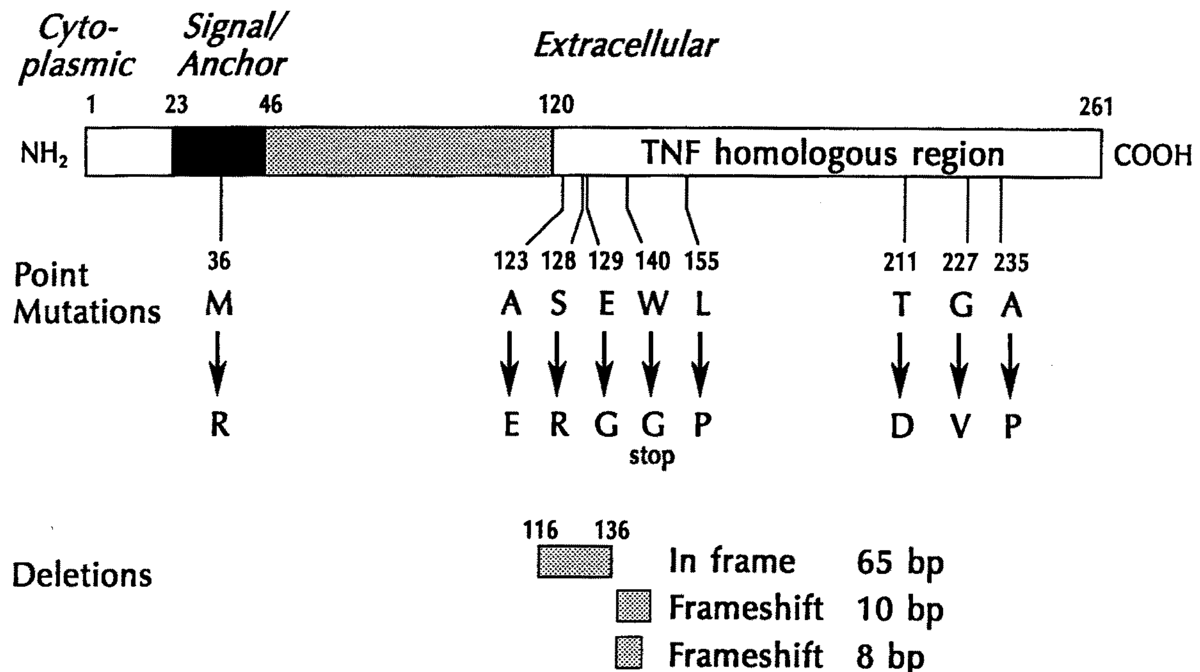

Fig. 1. Summary of amino acid changes found to date in the CD40L of X-linked HIGM patients.

Pneumocystis carinii infection, suggesting defective helper T cells. In addition, many patients exhibit a severe recurrent neutropenia (45, 46).

sIgM and $CD19^+$ or $CD20^+$ cells are present in HIGM patients, but B cells bearing mature isotypes are absent (47). *In vitro* T cell function tests are generally normal. T cell subpopulations are also considered normal. In the peripheral lymphoid tissue germinal centers may be diminished or absent.

The following paragraphs provide a summary of the findings of several laboratories which reported the association of defects in the CD40L gene and X-linked HIGM.

To date, nucleotide sequence results of the CD40L mRNA derived from 13 patients have been reported (24–27). Eight of these patients exhibit point mutations within the extracellular domain, three contain deletions, one contains a point mutation within the transmembrane domain, and one patient does not appear to have any changes in the coding region, suggesting a defect at the translational regulation level. The described point mutations are summarized in Fig. 1. Interestingly, many of these changes are relatively minor and might easily be considered naturally occurring polymorphisms. Subsequent experiments presented below have proven that the mutations are in fact deleterious and result in CD40L proteins which are blocked during intracellular transport or are expressed but do not bind to CD40. This suggests that the secondary and/or tertiary structure of the ligand is critical to its biological activity and very sensitive to alteration.

Flow cytometric analysis using a soluble CD40.Fc fusion protein on activated peripheral blood T cells (PB T) indicated that 12 of the 13 patients studied showed no CD40-Fc binding (24–27). One patient did show very weak, but reproducible, binding (24). In contrast, normal donor-activated PBL bound CD40-Fc well, indicating that any of the mutations listed in Fig. 1 are sufficient to interfere with biological activity.

The literature describing HIGM syndrome and its possible causes prior to the discovery of the CD40L defect was inconclusive in resolving whether HIGM patients carried a defect in their T cells or in their B cells (48, 49). To determine whether patient B cells could respond normally to wild-type CD40L, proliferative assays on four of the HIGM patients were performed. Cultures containing T-depleted peripheral blood mononuclear cells (PBMC) and CV-1/EBNA cells transfected with CD40L or with vector alone were analyzed for ^{3}H-TdR uptake. All four patients' B cell cultures showed levels of proliferation comparable to those of normal donor controls (24). Consistent with this, Aruffo *et al.* noted similar levels of patient and control proliferation in culture

systems containing PBMC, CD40 mAb, and IL-4 (25).

The diminished IgG, IgA, and IgE phenotype of X-linked HIGM patients suggested that their B cells should respond to wild-type CD40L and cytokines by producing mature forms of immunoglobulin. *In vitro* culture of normal PBMC in the presence of IL-4 results in detectable IgE secretion after 10 days (35). Cultures of HIGM PBMC with IL-4 or with IL-4 and CV-1/EBNA cells transfected with vector alone result in no detectable IgE secretion. However, coculture of patient PBMC with CV-1/EBNA cells expressing the CD40L does result in IgE secretion. Similar results are obtained from culture systems using CD40 mAb and IL-4 (7, 9, 10, 24). In addition, Korthauer *et al.* have demonstrated that HIGM patient B cells cultured in the presence of CD40L plus IL-10 can be induced to secrete IgG and IgA (26).

The combination of *in vitro* data with the known phenotype of X-linked HIGM patients clearly indicates that although CD40L functions *in vitro* as a potent mitogen for B cells, other mechanisms can and will function *in vivo* to allow proliferation and IgM secretion from B cells.

Additional PB T activation antigens were examined by flow cytometry to ensure that HIGM patient T cells could respond to polyclonal activation. In one instance, the p55 chain of the IL-2R was tested (24); in another, CD69 (25). In both cases, comparable surface expression was seen on HIGM-activated PB T as was evident on normal donor tissue. These data support the idea that the defect in the T cell population of HIGM patients is restricted to CD40L expression; it does not, however, prove it. In fact, recent unpublished findings (40, 50) which are described in the following section indicate that the CD40L can act as a costimulus for the proliferation of T cells. Thus, additional detailed studies are necessary before the full extent of defective CD40L expression is understood.

ACTIVITY OF THE CD40L ON MONOCYTES AND T CELLS

CD40 is known to be expressed on B cells, follicular dendritic cells, thymic epithelium, and some carcinoma cells. There is a single report of CD40 expression on monocytes (5); however, functional data or molecular studies have not been published. Recently, work in our lab has shown that elutriated primary peripheral blood monocytes can bind CD40 mAb (51). This binding is increased by culturing monocytes with GM-CSF, IL-3, or γIFN. Monocyte CD40 mRNA levels are also increased in response to these cytokines, suggesting that CD40L expression is regulated at the transcriptional level. Signaling through CD40 was shown to occur in studies where IL-6 and IL-8 production by monocytes was measured following culture with CV-1/EBNA cells transfected with CD40L or with vector alone. Reproducible secretion of these cytokines was detectable in response to CD40L in the absence of any costimulus. When cocultures containing CD40L and GM-CSF, IL-3, or γIFN were assayed, CD40L was shown to act as a costimulus for monocyte TNFα production and to enhance IL-6 secretion. IL-8 production was similarly enhanced using CD40L in conjunction with GM-CSF or IL-3 (51).

CD40L-stimulated monocytes were also tested for their tumoricidal activity. Culture of primary monocytes with CV-1/EBNA cells expressing CD40L in the absence of additional stimuli resulted in the induction of potent tumoricidal activity when assayed against the A375 melanoma line (51).

The stimulation of monocytes by CD40L suggests another level of complexity in the events surrounding antigen presentation. Activated $CD4^+$ T cells expressing CD40L may secrete cytokines which enhance CD40 expression on antigen presenting monocytes, which in turn produce monokines and exhibit tumoricidal activity or heterotypic cellular adherence characteristics, which then function directly or act as costimuli to T cells, perhaps in a type of feedback loop. Such studies suggest that the CD40–CD40L interaction between monocytes or macrophages and T cells is likely to play an important role in the effector function of these cell types. PB T cells have also been examined for their ability to respond to the CD40L (40). Enhanced expression of CD25 and the CD40L itself was seen in PB T cell cultures containing CV-1/EBNA cells expressing CD40L or recombinant soluble trimeric CD40L compared to control cultures. In addition, CD69 expression is increased in PB T cultures containing CD40L and CD3 mAb.

The CD40L also serves as a costimulus for PB T cell proliferation in cultures containing CD3 mAb or lectin. This proliferation occurs in both the $CD4^+$ and the $CD8^+$ populations and appears to be largely IL-2 independent. In other studies, the activity of the CD40L was comparable to that of IL-2 in the induction of cytotoxic T lymphocyte generation.

These studies, like those described for monocytes, suggest that the CD40L may play a more complicated, if subtle, role in the immune response than is currently appreciated.

DISCUSSION

Since the identification of a cDNA for the murine (mu) CD40L in late 1991, an impressive amount of information about both the biology of this molecule and B cells in general has been generated. The discovery that the CD40L is encoded on the X chromosome allowed an unexpected opportunity for investigators from a variety of disciplines to study the role of CD40–CD40L in T and B cell function, B cell maturation, and antibody production. In addition, a potential path for exploring the unknown biological activities resulting from this ligand–receptor interaction is possible through careful analysis of the clinical phenotype of HIGM patients.

The initial discovery that recombinant, membrane-bound CD40L is a potent inducer of B cell proliferation immediately suggested that CD40L likely played a role in B cell proliferation *in vivo.* It is fascinating, however, that in HIGM patients, relatively normal numbers of circulating B cells are seen in peripheral blood. This indicates that another as yet unidentified molecular interaction occurs *in vivo* which allows for B cell proliferation. The *in vitro* proliferative function of the CD40L will likely find use in systems which are currently being developed and are aimed at the production of human mAbs. Of interest also is the role of germinal centers in B cell proliferation and maturation. Germinal centers are virtually absent in HIGM patients, suggesting that the CD40–CD40L interaction is critical for their formation or maintenance. Clearly, however, functional B cells capable of producing antigen-specific antibody of the IgM isotype are found in normal levels in these patients (Ref. 28 and unpublished observations).

The finding that thymic epithelium produces GM-CSF in response to stimulation through surface CD40 raises the possibility that this interaction is at least partially responsible for the recurrent neutropenia seen in many HIGM patients. Another complexity is that the CD40L can function as a costimulus for cytokine secretion from monocytes and that T cells respond to CD40L by increasing its own surface expression. Thus, it is possible that a more subtle immune dysfunction occurs in these patients as a result of the absence of CD40 signaling on myeloid or lymphoid cells.

The variation in clinical phenotype provides a rich source of questions regarding CD40L interactions. For example, compensatory mechanisms, which may not be utilized in normal individuals, might play an important role in modulating this disease. The existence of HIGM patients who have reduced, but not absent, CD40L expression arising from such mechanisms as diminished CD40 binding and abnormal transcriptional regulation may also exhibit intermediate symptoms. These patients will no doubt provide valuable insight into this arm of the *in vivo* immune response.

Diagnosis and genetic counseling for HIGM are greatly improved by direct analysis of CD40L. Patients suspected of having HIGM may now be tested for expression of CD40L on activated T cells. Women who carry the defect may have reduced CD40L expression, but because of Lyonization, they may appear normal. A more reliable approach is direct mutation detection. The CD40L coding sequence is sufficiently short that direct sequencing from mRNA could be routinely applied. Once a mutation is detected, either allele-specific oligonucleotide hybridization or a mutation scanning technique (e.g., single-strand conformational polymorphism or heteroduplex formation) could be employed to genotype other family members. Finally, we have found that the CA repeat in the 3′ untranslated region of the CD40L mRNA is very polymorphic (estimated heterozygosity 70%) (52). This can be used in conventional linkage analysis for carrier testing and prenatal diagnosis. Until the CD40L gene structure is elucidated, the CA repeat, which can be amplified from genomic DNA, provides the best approach to genotyping of a fetus in early pregnancy.

At present, both *in vitro* and *in vivo* data clearly indicate that the CD40L, in conjunction with cytokines, is absolutely required for B cell isotype "switching." Although many details are still unknown, it is reasonable to assume that T cells become activated in response to antigen; secreting cytokines and upregulating a variety of cell surface molecules. One of these, CD40L, interacts with its cognate expressed on the surface of B cells, monocytes, T cells, and some epithelial cells. Subsequent cytokine production ensues, and resting B cells are activated to proliferate and produce the more mature immunoglobulin isotypes.

The next few years will yield unprecedented amounts of information on humoral immunity, not the least of which will be contributions made by experiments using soluble CD40L as an antagonist, or possibly as an adjuvant, passive transfer studies using neutralizing antibody against the CD40L, analysis of CD40L single-gene disruptions in mice, and comprehensive examination of the CD40–CD40L cognate interactions.

REFERENCES

1. Stamenkovic I, Clark EA, Seed B: A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. EMBO J 8:1403–1410, 1989
2. Smith CA, Davis T, Anderson D, Solam L, Beckmann MP, Jerzy R, Dower SK, Cosman D, Goodwin RG: A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins. Science 248:1019–1023, 1990
3. Mallett S, Barclay AN: A new superfamily of cell surface proteins related to the nerve growth factor receptor. Immunol Today 12:220–223, 1991
4. Dürkop H, Latza U, Hummel M, Eitelbach F, Seed B, Stein H: Molecular cloning and expression of a new member of the nerve growth factor receptor family that is characteristic for Hodgkin's disease. Cell 68:421–427, 1992
5. Clark EA: CD40: A cytokine receptor in search of a ligand. Tissue Antigens 36:33–36, 1990
6. Banchereau J, de Paoli P, Valle A, Garcia E, Rousset F: Long term human B cell lines dependent on interleukin-4 and antibody to CD40. Science 251:70–72, 1991
7. Jabara HH, Fu SM, Geha RS, Vercelli D: CD40 and IgE: Synergism between anti-CD40 monoclonal antibody and interleukin-6 in the induction of IgE synthesis by highly purified human B cells. J Exp Med 172:1861–1864, 1990
8. Rousset F, Garcia E, Banchereau J: Cytokine-induced proliferation and immunoglobulin production of human B lymphocytes triggered through their CD40 antigen. J Exp Med 173:705–710, 1991
9. Zhang K, Clark EA, Saxon A: CD40 stimulation provides and IFN-γ-independent and IL-4-dependent differentiation directly to human B cells for IgE production. J Immunol 146:1836–1842, 1991
10. Gascan H, Gauchat J-F, Aversa G, van Vlasselaer P, de Vries JE: Anti-CD40 monoclonal antibodies or $CD4^+$ T cell clones and IL-4 induce IgG4 and IgE switching in purified human B cells via different signalling pathways. J Immunol 147:8–13, 1991
11. Rousset F, Garcia E, Defrance T, Peronne C, Vezzio N, Hsu DH, Kastelein R, Moore KW, Banchereau J: Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA 89:1890–1893, 1992
12. Shapira SK, Vercelli D, Jabara HH, Fu SM, Geha RS: Molecular analysis of the induction of immunoglobulin E synthesis in human B cell by interleukin 4 and engagement of CD40 antigen. J Exp Med 175:289–292, 1992
13. Liu YJ, Joshua DE, Williams GT, Smith CA, Gordon J, MacLennon ICM: Mechanism of antigen-driven selection in germinal centres. Nature (London) 342:929–931, 1989
14. Liu YJ, Cairns JA, Holder MJ, Abbot SD, Jansen KU, Bonnefoy JY, Gordon J, Mac Lennan IC: Recombinant 25-kDa CD23 and interleukin 1α promote the survival of germinal center B cells: evidence for bifurcation in the development of centrocytes rescued from apoptosis. Eur J Immunol 21:1107–1114, 1991
15. Liu YJ, Mason DY, Johnson GD, Abbot S, Gregory CD, Hardie DL, Gordon J, Mac Lennan IC: Germinal center cells express bcl-2 protein after activation by signals which prevent their entry into apoptosis. Eur J Immunol 21:1905–1910, 1991
16. Galy AH, Spits H: CD40 is functionally expressed on human thymic epithelial cells. J Immunol 149:775–782, 1992
17. Gordon J, Millsum MJ, Guy GR, Ledbetter JA: Resting B lymphocytes can be triggered directly through the CD_w40 (Bp50) antigen. J Immunol 140:1425, 1988
18. Barrett TB, Shu G, Clark EA: CD40 signaling activates CD11a/CD18 (LFA-1)-mediated adhesion in B cells. J Immunol 146:1722, 1991
19. Spriggs MK, Armitage RJ, Strockbine L, Clifford KN, Macduff BM, Sato TA, Maliszewski CR, Fanslow WC: Recombinant human CD40 ligand stimulates B cell proliferation and Immunoglobulin E secretion. J Exp Med 176:1543–1550, 1992
20. Graf D, Korthauer U, Mages HW, Senger G, Kroczek RA: Cloning of TRAP, a ligand for CD40 on human T cells. Eur J Immunol 22:3191–3194, 1992
21. Hollenbaugh D, Grosmaire LS, Kullas CD, Chalupny NJ, Braesch-Andersen S, Noelle RJ, Stamenkovic I, Ledbetter JA, Aruffo A: The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity. EMBO J 11:4313–4321, 1992
22. Armitage RJ, Fanslow WC, Strockbine L, Sato TA, Clifford KN, Macduff BM, Anderson DM, Gimpel SD, Davis-Smith T, Maliszewski CR, Clark EA, Smith CA, Grabstein KH, Cosman D, Spriggs MK: Molecular and biological characterization of a murine ligand for CD40. Nature (London) 357:80–82, 1992
23. Farrah T, Smith CA: Emerging cytokine family. Nature (London) 358:26, 1992
24. Allen RC, Armitage RJ, Conley ME, Rosenblatt H, Jenkins NA, Copeland NG, Bedell MA, Edelhoff S, Disteche CM, Simoneaux DK, Fanslow WC, Belmont J, Spriggs MK: CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. Science 259:990–993, 1993
25. Aruffo A, Farrington M, Hollenbaugh D, Li X, Milatovich A, Nonoyama S, Bajorath J, Grosmaire LS, Stenkamp R, Neubauer M, Roberts RL, Noelle RJ, Ledbetter JA, Francke U, Ochs HD: The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. Cell 72:291–300, 1993
26. Korthauer U, Graf D, Mages HW, Briere F, Padayachee M, Malcolm S, Ugazio AG, Notarangelo LD, Levinsky RJ, Kroczek RA: Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper IgM. Nature (London) 361:539–541, 1993

27. DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G: CD40 ligand mutations in X-linked immunodeficiency with hyper-IgM. Nature (London) 361:541–543, 1993
28. Geha RS, Hyslop N, Alami S, Farah F, Schneeberger EE, Rosen FS: Hyper immunoglobulin M immunodeficiency. (Dysgammaglobulinemia). Presence of immunoglobulin M-secreting plasmacytoid cells in peripheral blood and failure of immunoglobulin M-immunoglobulin G switch in B-cell differentiation. J Clin Invest 64:385–391, 1979
29. Noelle RJ, McCann J, Marshall L, Bartlett WC: Cognate interactions between helper B cells and B cells. III. Contact-dependent, lymphokine-independent induction of B cell cycle entry by activated helper T cells. J Immunol 143:1807, 1989
30. Noelle RJ, Snow EC: T helper cell-dependent B cell activation. FASEB J 5:2770–2706, 1991
31. Noelle RJ, Daum J, Bartlett WC, McCann J, Shepherd DM: Cognate interactions between helper T cells and B cells. V. Reconstitution of helper T cell function using purified plasma membranes from activated Th1 and Th2 helper T cells and lymphokines. J Immunol 146:1118–1124, 1990
32. Brian AA: Stimulation of B-cell proliferation by membrane-associated molecules from activated T cells. Proc Natl Acad Sci USA 85:564–568, 1988
33. Sekita K, Straub C, Hoessli D, Zubler RH: B cell-stimulating activity of lymphoid cell membrane fractions. Eur J Immunol 18:1405–1410, 1988
34. Hodgkin PD, Yamashita LC, Coffman RL, Kehry MR: Separation of events mediating B cell proliferation and Ig production by using T cell membranes and lymphokines. J Immunol 145:2025–2034, 1990
35. Fanslow WC, Anderson D, Grabstein KH, Clark EA, Cosman D, Armitage RJ: Soluble forms of CD40 inhibit biological responses of human B cells. J Immunol 149:655–660, 1992
36. McMahan CJ, Slack JL, Mosley B, Cosman D, Lupton SD, Brunton LL, Grubin CE, Wignall JM, Jenkins NA, Brannan CI, Copeland NG, Huebner K, Croce CM, Cannizzarro LA, Benjamin D, Dower SK, Spriggs MK, Sims JE: A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. EMBO J 10:2821–2832, 1991
37. Armitage RJ, Macduff BM, Spriggs MK, Fanslow WC: Human B cell proliferation and Ig secretion induced by recombinant CD40 ligand are modulated by soluble cytokines. J Immunol 150:3671–3680, 1993
38. Andersson J, Schreier MH, Melchers F: T-cell-dependent B-cell stimulation is *H-2* restricted and antigen dependent only at the resting B-cell level. Proc Natl Acad Sci USA 77:1612–1616, 1980
39. Peitsch MC, Jongeneel CV: A 3-D model for the CD40 ligand predicts that it is a compact trimer similar to the tumor necrosis factors. Int Immunol 5:233–238, 1993
40. Armitage RJ, Tough TW, Macduff BM, Fanslow WC, Spriggs MK, Ramsdell F, Alderson MR: CD40 ligand is a T-cell growth factor. Eur J Immunol (in press), 1993
41. Copeland NG, Jenkins NA: Development and applications of a molecular genetic linkage map of the mouse genome. Trends Genet 7:113–118, 1991
42. Mensink EJ, Thompson A, Sandkuyl LA, Kraakman ME, Schot JD, Espanol T, Schuurman RK: X-linked immunodeficiency with hyperimmunoglobulinemia M appears to be linked to the DXS42 restriction fragment length polymorphism locus. Hum Genet 76:96–99, 1987
43. Padayachee M, Feighery C, Finn A, McKeown C, Levinsky RJ, Kinnon C, Malcolm S: Mapping of the X-linked form of hyper-IgM syndrome (HIGM1) to Xq26 by close linkage to HPRT. Genomics 14:551–553, 1992
44. Rosen FS, Kevy SV, Merler E, Janeway CA, Gitlin D: Recurrent bacterial infections and dysgammaglobulinemia: Deficiency of 7S gamma-globulins in the presence of elevated 19S gamma-globulins. Pediatrics 28:182–195, 1961
45. Notarangelo LD, Duse M, Ugazio AG: Immunodeficiency with hyper-IgM (HIM). Immunodefic Rev 3:101–121, 1992
46. Conley ME: Molecular approaches to analysis of X-linked immunodeficiencies. Annu Rev Immunol 10:215–238, 1992
47. Mayer L, Kwan SP, Thompson C, Ko HS, Chiorazzi N, Waldmann T, Rosen F: Evidence for a defect in "switch" T cells in patients with immunodeficiency and hyperimmunoglobulinemia M. N Engl J Med 314:409–413, 1986
48. Levitt D, Haber P, Rich K, Cooper MD: Hyper IgM immunodeficiency. A primary dysfunction of B lymphocyte isotype switching. J Clin Invest 72:1650–1657, 1983
49. Hendriks RW, Kraakman ME, Craig IW, Espanol T, Schuurman RK: Evidence that in X-linked immunodeficiency with hyperimmunoglobulinemia M the intrinsic immunoglobulin heavy chain class switch mechanism is intact. Eur J Immunol 20:2603–2608, 1990
50. Fanslow WC, Clifford KY, Seaman M, Alderson MR, Spriggs MK, Armitage RJ, Ramsdell F: Recombinant CD40-ligand exerts potent biological effects on T cells. J Immunol (in press) 1993
51. Alderson MR, Armitage RJ, Tough TW, Strockbine L, Fanslow WC, Spriggs MK: CD40 expression by human monocytes: Regulation by cytokines and activation of monocytes by the ligand for CD40. J Exp Med 178:669, 1993
52. Allen RC, Spriggs MK, Belmont JW: Dinucleotide repeat polymorphism in the human CD40 ligand gene. Hum Mol Genet 2:828, 1993