[Frontiers in Bioscience 2, d1-11 January 1, 1997]

IMMUNE REGULATION BY CD40-CD40-L INTERACTIONS

Cees van Kooten and Jacques Banchereau

Dept of Nephrology, Leiden University Hospital, Leiden, the Netherlands and Schering-Plough, Laboratory for Immunological Research, Dardilly, France

TABLE OF CONTENTS

1. ABSTRACT

CD40 is a cell surface receptor, which belongs to the TNF-R family, and which was first identified and functionally characterized on B lymphocytes. However, in recent years it has become clear that CD40 is expressed much broader, 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 main emphasis on 1) structure and expression of CD40 and its ligand; 2) CD40 signal transduction; 3) *in vitro* function of CD40 on different cell types; 4) *in vivo* functions of CD40/CD40-L interactions.

2. INTRODUCTION

12 years after the identification of the CD40 antigen through monoclonal antibodies, a wealth of information has been generated, which identify CD40 and its ligand as critical entities in the regulation of immune responses.

Received 11/14/96; Accepted 12/02/96

1 To whom correspondence should be addressed, at Schering Plough Laboratory for Immunological Research, 27, chemin des Peupliers, BP 11, 69571 Dardilly, France. Tel #: (33)4.72.17.27.00; Fax #: (33)4.78.35.47.50. E-mail: J Banchereau: LIR@dial.oleane.com, Cv Kooten: kooten@ nephrology. medfac.leidenuniv.nl

The interest on this molecule is increasing as shown by a Medline search for the textword "CD40", which yielded 6 hits in 1989, 18 in 1990-mid1991, 71 in mid1991-1992, 110 in 1993, 210 in 1994-mid1995 and 248 in mid1995-mid1996, respectively.

Early studies, concentrated on the role of CD40 in B cell physiology, have culminated with the finding that a defective CD40-CD40-L interaction (by mutations in the CD40-L gene), is actually the cause for the X-linked immunodeficiency hyper-IgM syndrome. Since, the availability of specific molecular tools and the generation of both CD40 and CD40L knockout mice, have extended the research in much broader ways. Recent investigations have resulted in an explosion of data concerning: 1) the structure and expression of CD40 and its ligand; 2) the signal transduction mechanisms of CD40; 3) the functional expression of CD40 on cells other than B cells; 4) the *in vivo* role of CD40-CD40-L interactions. As these later developments will be the focus of the present review, readers are referred to several other recent reviews (1-6).

3. IMMUNE REGULATION BY CD40-CD40-L INTERACTIONS

3.1. Structure and expression of CD40 and its ligand

The CD40 antigen is a 45-50 kDa glycoprotein of 277 AA, which belongs to the Tumor Necrosis Factor Receptor family (7). The 193 AA extracellular domain is composed of four

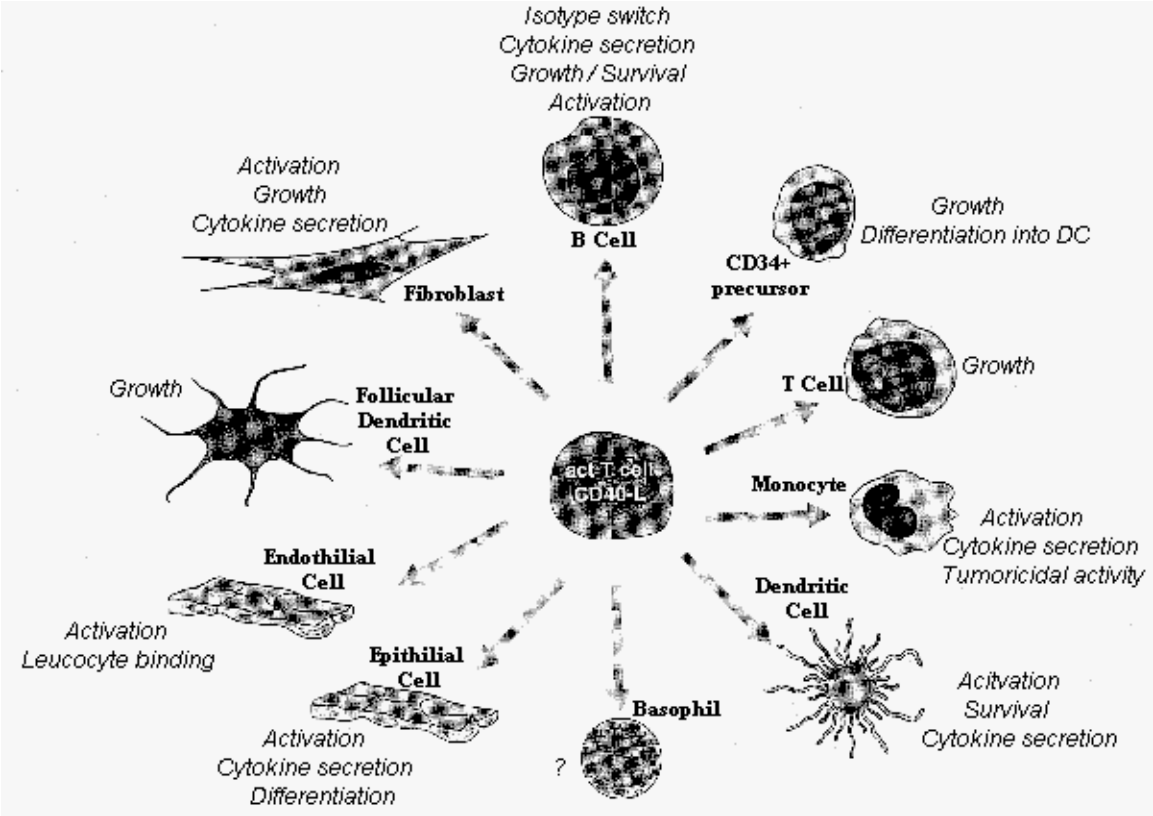

Figure 1: Functional consequences of CD40 triggering on CD40 expressing cell types. Activation includes increase in cell size and alteration of phenotype.

imperfect repeats of ~ 40 residues, anchored by a superimposable pattern of six cysteines. This organization is found in the other members of the family including: the p75 low-affinity Nerve Growth Factor Receptor; the p55/CD120a and p75/CD120b receptors for Tumor Necrosis Factor; the receptor (TNFR rp) for the Iymphotoxin α / Iymphotoxin β membrane complex; CD27; CD30; OX 40; 4-1-BB; FAS/CD95; two viral homologs of the TNF receptors. The X-ray crystal structure of the complex formed by soluble binding domains of p55 TNFR and a Iymphotoxin a trimer suggests that CD40 looks like a slightly bend rod. Three CD40 subunits are likely to engage the CD40-L trimer and scrupulously avoid contact between each other. The mouse CD40 gene, composed of nine exons that span a 16.3 kb of genomic DNA, is located on the distal region of chromosome 2 which is syntenic to human chromosome 20q11-q13 where the human CD40 gene is located.

CD40 is expressed by multiple cell types. In the hematopoietic system, it is expressed on $CD34^+$ hematopoietic progenitors, B cell progenitors, mature B Iymphocytes, plasma cells, monocytes, dendritic cells, eosinophils, basophils and on some T Iymphocytes. CD40 is also expressed on non-hematopoietic cells such as endothelial cells, fibroblasts and epithelial cells (5)(figure 1).

In 1992, expression cloning using a CD40-Fc fusion protein allowed the isolation of a CD40-ligand (CD40-L) from activated T cells (8). The human CD40-L is a polypeptide of 261 AA including a 215 AA extracellular domain with five cysteins. CD40-L is a member of the Tumor Necrosis Factor family that includes TNFα, LTα, LTβ, CD27-L/CD70, CD30-L, 4-1BB-L, OX40-L, FAS-L and TRAIL/APO2 (7, 9, 10). The gene for CD40-L is located on the X-chromosome at position q26.3-q23.1. It spans over 12-13 kb and consists of five exons. CD40-L is expressed on $CD4^+$ and $CD8^+$ T cells readily after activation through pathways that are inhibited by Cyclosporin A (11). Basophils, eosinophils and activated B Iymphocytes have also been reported as expressing CD40-L. In patients with SLE, CD40L has been found hyperexpressed on both T and B lymphocytes (12, 13).

3.2. CD40 signal transduction

Like all other members of the TNF-R family, CD40 has no kinase domain and no known consensus sequence for binding to kinases. Yet, CD40 ligation activates protein-tyrosine kinases, including *Lyn* and *Syk* and induces the tyrosine

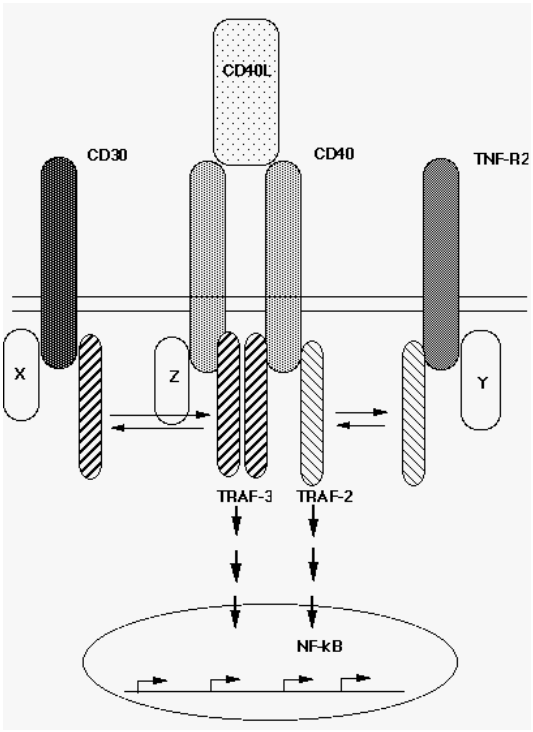

Figure 2: Schematic representation of molecules involved in CD40 signal transduction. X,Y and Z represent as yet unidentified molecules associated with the signalling complexes of TNF-R members. TRAF2 and TRAF3, which are indicated by hatched figures, associate with CD40, but are also shared by other members of TNF-R family (ie CD30 and TNF-R2). For more details, readers are referred to the text.

phosphorylation of multiple substrates including phosphatidylinositol 3-kinase and phospholipase Cγ2. The phosphorylation of the latter is consistent with the anti-CD40 induced IP3 production. The inhibitory effects of protein-tyrosine kinase inhibitors and of CD45 crosslinking demonstrate the functional relevance of the CD40-induced tyrosine phosphorylation (14). CD40 ligation also appears to activate the serine-threonine protein kinases and most particularly the stress-activated protein kinases (SAPK, also known as JNK for c-jun NH2-terminal kinase)(15, 16). Finally, these different activation pathways result in the activation of various transcription factors including NF-κB, NF-κB-like molecules (such as p50, p65 (rel A), cRel), c-jun and NF-AT.

In recent years it has been established that members of the TNF- family associate intracellularly with different families of signalling molecules, including the 'death domain' family and the TRAF family (17). With the two-hybrid system, such protein-protein interactions have been demonstrated for TNF-R1, TNF-R2, Fas/CD95, CD30 and CD40. Interestingly, although there is no cross reactivity between the extracellular ligands of the TNF-R family (with the exception of TNF and LT), the intracellular ligands seem to be much more promiscuous and form a complex network of homo- and hetero-dimers (figure 2).

CD40 interacts with TRAF3 (TNF-R Associated Factor-3), also identified under the name CRAF1, CD40bp, LAP1 and CAP1 (18-21). TRAF3 is a 62 kD intracellular protein which is expressed in almost all cell types. The protein contains several functional domains which might be involved in signal transduction (RING finger domain, Isoleucine zipper, Zinc finger) or which play a role in protein-protein interactions either to associate with CD40 or to form homo- / heterodimers. TRAF3 appears to be essential for CD40 signalling inasmuch as a mutant CD40 with a point mutation at position 234 Thr > Ala, which is functionally inactive, does not bind TRAF3 (19). TRAF3 has been demonstrated to associate with CD30 (22). Interestingly, TRAF3 also binds to the EBV (Epstein Barr Virus) transforming gene product LMP1 (latent infection membrane-protein-1), which indicates that EBV utilizes the CD40 signalling pathway to activate and immortalize B lymphocytes (20).

In addition, CD40 has been demonstrated to associate with TRAF2, a molecule which also associates with TNF-R2. The induction of NF-κB activation via CD40 crosslinking (and also via TNF-

R2) could be attributed to TRAF2 signalling (23), because a truncated TRAF2 protein, lacking an amino-terminal RING finger domain behaves as a dominant negative inhibitor. Finally, a novel 23 kD cell surface protein has been demonstrated to associate with CD40, but the functional relevance of this protein awaits more experimental data (24).

3.3. Functions of CD40 *in vitro*.

3.3.1. B Iymphocytes

CD40 ligation activates resting B cells as shown by increase in size and expression of new surface molecules involved in homotypic and heterotypic aggregation (CD23, VLA-4), as well as T cell costimulation (CD80/CD86). Furthermore, CD40-activated B cells secrete a panel of cytokines which may act as autocrine and paracrine growth and differentiation factors (25, 26).

CD40-activated B cells enter into proliferation which is further stimulated by addition of cytokines such as IL4, IL13 or IL10 and their combination (1). Cytokines can also induce CD40-activated B cells to secrete immunoglobulins, with IL10 inducing the secretion of large quantities of Igs as a consequence of induced plasma cell differentiation. In addition to being a differentiation factor, IL10 acts as a factor inducing switch towards IgG1 and IgG3, as demonstrated at the molecular level by the appearance of 'switch circles (27). Combination of IL10 and TGFβ induce IgA production, and IL4/lL13 induce CD40-activated B cells to switch towards IgG4 and IgE.

Interestingly, CD40 activation of B cells also results in the induction of Fas expression, and renders cells susceptible to Fas-induced apoptosis (28-30). In fact, together with BCR crosslinking, these three receptors generate a complex network of positive and negative signals, where the response of the B cell, activation or death, is determined by its differentiation stage (31-33). Importantly, dual triggering of resting B Iymphocytes through their CD40 and antigen receptor induces a phenotype characteristic of cells from germinal centers (34, 35), the anatomical site where B cells undergo somatic mutation, selection, isotype switching and become either plasma or memory cells. Prolonged triggering of CD40 skews the maturation of B cells into memory cells while interruption of CD40 signalling allows plasma cell differentiation (36, 37).

Although most normal and malignant B cells proliferate in response to CD40 engagement, plasma cells appear unresponsive (38). Furthermore, CD40 ligation appears to inhibit the proliferation of diffuse B cell Iymphomas, thus illustrating a possible negative role of CD40 on cell proliferation (39). A similar observation of negative signalling has been made in several transformed cell lines of both mesenchymal and epithelial origin (40).

3.3.2. Monocytes and dendritic cells

The expression of CD40 on professional antigen presenting cells like monocytes and dendritic cells is well established now. In addition, the functional role of CD40 on these cell types is becoming well documented both by *in vitro* and *in vivo* experiments (41, 42). Low spontaneous expression of CD40 is detected on freshly isolated monocytes, which can be upregulated by cytokines such as GM-CSF, IL3 and IFNγ (43). In contrast, CD40 is expressed at high levels on dendritic cells isolated from different tissues or generated *in vitro* by culturing hematopoietic progenitors (44-49). Interestingly, CD40 expression has also been detected on monocytes infiltrating brain lesions of patients with Multiple Sclerosis (50).

CD40 ligation of monocytes and dendritic cells results in the secretion of multiple proteins including cytokines such as IL1, IL6, IL8, IL10, IL12, TNFα, MiP1α (43, 45, 51, 52), as well as enzymes such as matrix metalloproteinase (MMP)(53). Importantly, the secretion of IL12 allows a skewing of T cell maturation towards the Th1 pathway (52, 54-57). It has been suggested that CD40 specifically induces the expression of p40 (54). In addition, CD40 ligation considerably alters the phenotype of these APCs by upregulating the expression of costimulatory molecules such as CD54 (ICAM-1), CD58/LFA-3, CD80/B7-1, CD86/B7-2 (45, 46, 49, 57, 58). Interrupting CD40/CD40-L interactions during T cell/dendritic cell cocultures results in reduced T cell proliferation (47, 59), possibly as a consequence of both altered CD40 signalling to the APCs (reduced expression of costimulatory membrane molecules and cytokines) and altered CD40-L signalling to the T cells (figure 3).

Ligation of CD40 enhances the survival of dendritic cells and monocytes (45, 49, 60, 61). Of note, CD40 ligation of $CD34^+$ hematopoietic progenitors induces their proliferation and differentiation into cells with prominent dendritic cell attributes (62).

The importance of this receptor-ligand pair for the cellular immune response, has been demonstrated by the diminished immunity against several pathogens in CD40 and CD40L knockout mice (see section 3.4). In keeping with this, CD40 ligation turns on monocyte tumoricidal activity as well as NO synthesis (63, 64).

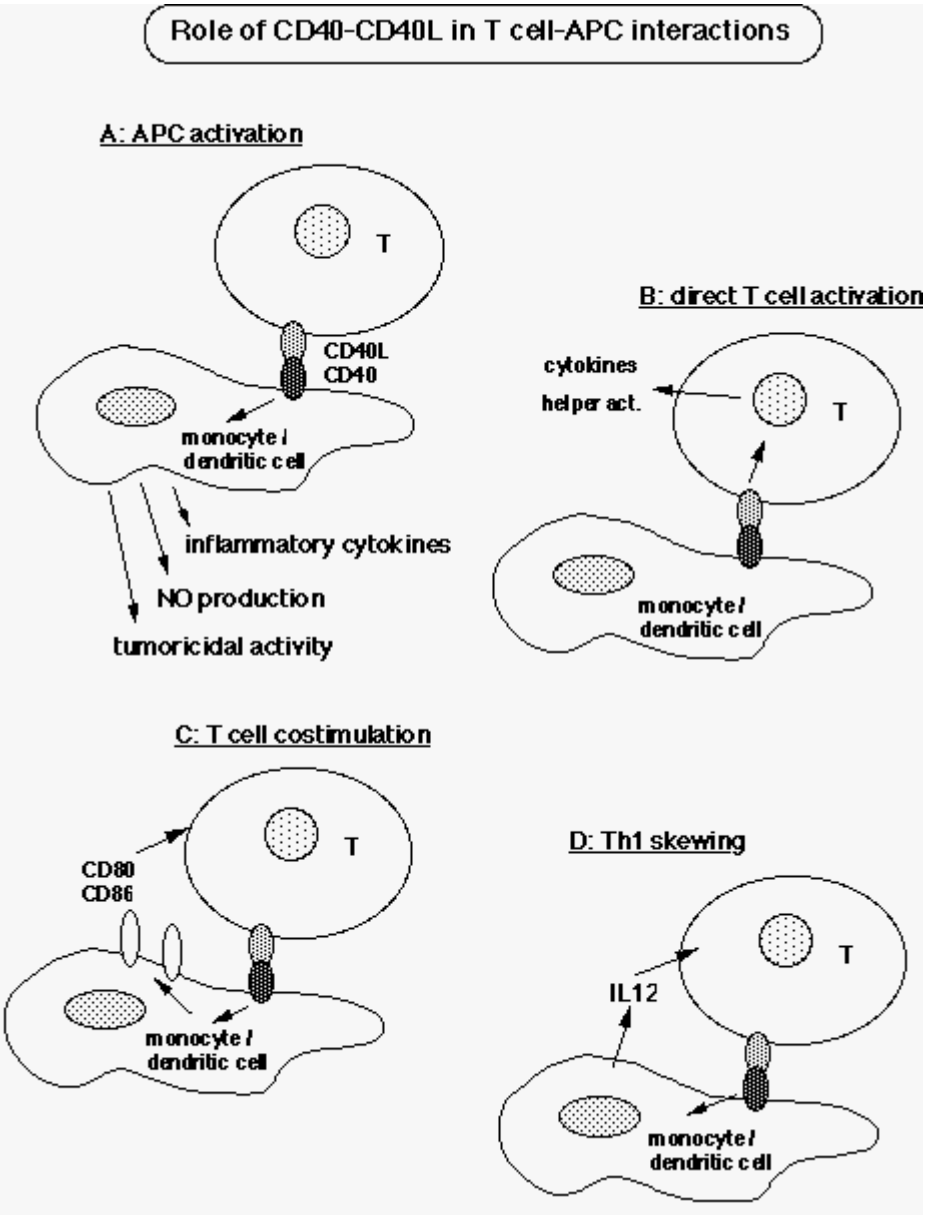

Figure 3: Functional consequences of CD40-CD40L interactions between activated T cells and Antigen Presenting Cells (monocytes and dendritic cells). The functional consequences of CD40 crosslinking on B cells are not included in this figure. Although most pathways are operational at the same time, for reasons of clarity, processes are separated in: A) activation of the APC; B) direct T cell activation via CD40L; C) T cell costimulation via costimulatory molecules; D) Th1 skewing via cytokine production.

3.3.3. Endothelial cells, epithelial cells and fibroblasts.

Immunohistology performed on various tissue sections shows that anti-CD40 antibodies stain vascular endothelium, epidermal basal membrane, scattered fibroblasts, thymic epithelium and follicular dendritic cells. Recently, CD40 expression was demonstrated in lesions of Kaposi's Sarcoma, as well as on vascular endothelium in areas adjacent to the tumors (65). Consistently, primary lines of endothelial cells, thymic and kidney tubular epithelial cells, keratinocytes, skin and synovial fibroblasts as well as follicular dendritic cells express CD40 at a relatively low density (5, 66). Yet, these adherent cells express a functional CD40 whose ligation induces phenotypic alterations, cytokine secretion as well as stimulation or inhibition of proliferation. In particular, endothelial cells display marked upregulation of CD54/lCAM-1, CD106/VCAM-1 and CD62E/E-Selectin, thus, resulting in increased ability to bind leukocytes (67-69). On the other hand, endothelial cells are also capable to enhance the expression of CD40L on activated T cells, probably by a CD2/LFA3 mediated pathway (70).

3.4. *in vivo* functions of CD40/CD40-L interactions.

3.4.1. Hyper IgM Syndrome

The first demonstration of the critical role of CD40/CD40-L interactions *in vivo* came from the discovery that the hyper IgM syndrome, an X-linked immunodeficiency, is due to a genetic alteration of the CD40-L (71). This disease is characterized by a severe impairment of T cell dependent antibody responses with no B cell memory and no circulating IgG, IgA and IgE. Initial experiments performed with antagonists to CD40/CD40-L interactions such as antibodies to CD40-L or CD40-Fc fusion proteins demonstrated impairment of B cell memory (72). The generation of CD40 and CD40-L knockout mice confirmed and further extended these observations and revealed a phenotype comparable to that of the patients suffering from the hyper IgM syndrome. In particular, these mice display decreased IgM responses to thymus dependent antigens, no antigen specific IgG1, IgA and IgE but normal T and B Iymphocyte numbers. As expected, these mice respond normally to thymus independent antigens with increased IgM and IgG3 levels.

Patients with hyper-IgM syndrome have an enhanced susceptibility to opportunistic infections, such as *Pneumocystis carinii* pneumonia and *Cryptosporidium* diarrhea. This indicates a role for CD40-CD40-L interactions in cell-mediated immune responses. Indeed, CD40-L knockout mice display a considerable impairment of antigen specific T cell priming (73) and appear particularly susceptible to Leishmania infection (74-76). This most likely results from a defective Th1 response which is related to an impaired production of IL12 by antigen presenting cells.

3.4.2. Formation of germinal centers

Germinal centers are the anatomical sites in lymphoid organs where isotype switching and somatic mutations are initiated (77). In a recent study, it was demonstrated that patients with the hyper-IgM syndrome lack germinal centers in their lymphoid organs (78). In accordance, somatic mutations within VDJ transcripts could not be detected in the circulating B cells from 5/6 patients (79, 80). Interestingly, the patient who had almost normal levels of somatic mutations, displayed a mutation within the transmembrane region of CD40L, which results in a very transient CD40L expression on activated T cells (80). These data suggest that a minimal CD40L expression is sufficient to allow the activation of events leading to the introduction of somatic mutations *in vivo*. However, CD40 activation of B cells per se, is not sufficient to induce somatic mutations *in vitro* (81), while activated T cells can do so (82)(Razajanaona et al. and Denepoux et al., unpublished data).

Studies with a CD40-Fc fusion protein have highlighted the important role of T cell signalling through CD40 ligand in the development of helper function. In particular, administration of soluble CD40 *in vivo* to CD40 knockout mice initiates germinal center formation and T cells primed in the absence of CD40 are unable to help normal B cells to class switch and to form germinal centers (83). In this context, ligating CD40 ligand of human activated T cells considerably enhances their production of IL4 (84, 85).

3.4.3. CD40/CD40-L: treatment of autoimmunity

Administration of antibodies to CD40-L has been shown to prevent the establishment of autoimmune symptoms in various murine models including: 1) collagen type II-induced arthritis; a model for human rheumatoid arthritis; 2) lupus nephritis in lupus prone mice that represent models for the systemic lupus erythematosus; 3) proteolipoprotein induced experimental encephalomyelitis; a model of human multiple sclerosis. Importantly, in this latter case, the antibody could induce an important reduction of the disease even when administered after onset. Consistently, activated helper T cells expressing CD40-L surface protein are detected in multiple sclerosis patient brain sections where CD40 bearing antigen presenting cells can be found.

3.4.4. CD40/CD40-L: induction of transplantation tolerance

Administration of anti-CD40-L antibodies prevents the development of graft versus host disease (GVHD) that occurs as a major complication of allogeneic bone marrow transplantation (86). This treatment affects both the acute GVHD, essentially mediated by cytotoxic T cells, and the chronic GVHD, mainly due to the polyclonal B cell activation and the production of self reactive antibodies. The addition of anti-CD40-L antibody appears to considerably enhance the tolerogenic effect of B cells (87). Furthermore, a combination of allogeneic B cells and anti-CD40-L antibody considerably decreases host reactivity of both $CD4^+$ and $CD8^+$ T Iymphocytes thereby allowing efficient transplantation of allogeneic pancreatic β islet cells (88). Anti-CD40-L antibodies markedly extend the survival of cardiac allografts in both naive and sensitized hosts when administered at the time of transplantation (89). More importantly, even long term acceptance of skin and cardiac allografts can be obtained by a simultaneous blocking of the CD40 and CD28 pathways (90).

4. CONCLUDING REMARKS

In recent years, the focus of CD40 research has been shifted from the study of B cell regulation (humoral immunity), to the study of a general

regulator of immune and inflammatory processes. Especially the finding of CD40 expression on activated endothelium, has important clinical implications, as it places this molecule in the centre of (chronic) inflammation, transplantation, tumor metastasis, angiogenesis and normal leukocyte trafficking. Thus, multiple disease states appear to be improved by interruption of CD40/CD40-L interactions. This is presently accomplished by preventing the association of the receptor with its ligand using specific antibodies or soluble receptor molecules. However, for clinical applications, such reagents may not prove useful therapeutic entities. The identification of small synthetic chemical agents preventing the interaction of CD40 with its ligand would be of interest. Alternatively, by unravelling the signal transduction pathways of CD40 in different target cells, pharmacologic agents may be developed which will specifically block the intracellular pathways turned on after ligation of either CD40 or CD40-L. The recent identification of a dominant negative inhibitor of CD40 activation might represent a first step in this direction.

5. REFERENCES

1 J. Banchereau, F. Bazan, D. Blanchard, F. Briere, J. P. Galizzi, C. van Kooten, Y. J. Liu, F. Rousset & S. Saeland: The CD40 antigen and its ligand. [Review]. *Annu Rev Immunol* 12, 881-922 (1994)

2 E. A. Clark & J. A. Ledbetter: How B and T cells talk to each other. [Review]. *Nature* 367, 425-8 (1994)

3 D. Hollenbaugh, H. D. Ochs, R. J. Noelle, J. A. Ledbetter & A. Aruffo: The role of CD40 and its ligand in the regulation of the immune response. *Immunol Rev* 138, 23-37 (1994)

4 R. J. Noelle: CD40 and its ligand in host defense. [Review]. *Immunity* 4, 415-9 (1996)

5 C. Van Kooten & J. Banchereau: CD40-CD40 ligand: a multifunctional receptor-ligand pair. *Adv Immunol* 61, 1-77 (1996)

6 T. M. Foy, A. Aruffo, J. Bajorath, J. E. Buhlmann & J. Noelle: Immune regulation by CD40 and its ligand gp39. *Annu Rev Immunol* 14, 591-617 (1996)

7 C. A. Smith, T. Farrah & R. G. Goodwin: The TNF receptor superfamily of cellular and viral proteins: Activation, costimulation and death. *Cell* 76, 959-962 (1994)

8 R. J. Armitage, W. C. Fanslow, L. Strockbine, T. A. Sato, K. N. Clifford, B. M. Macduff, D. M. Anderson, S. D. Gimpel, T. Davis-Smith, C. R. Maliszewski, E. A. Clark, C. A. Smith, K. H. Grabstein, D. Cosman & M. K. Spriggs: Molecular and biological characterization of a murine ligand for CD40. *Nature* 357, 80-2 (1992)

9 S. R. Wiley, K. Schooley, P. J. Smolak, W. S. Din, C. P. Huang, J. K. Nicholl, G. R. Sutherland, T. Davis-Smith, C. Rauch, C. A. Smith & R. G. Goodwin: Identification and charaterization of a new member of the TNF family that induces apoptosis. *Immunity* 3, 673-682 (1995)

10 R. M. Pitti, S. A. Marsters, S. Ruppert, C. J. Donahue, A. Moore & A. Ashkenazi: Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. *J Biol Chem* 271, 12687-12690 (1996)

11 R. Fuleihan, N. Ramesh, A. Horner, D. Ahern, P. J. Belshaw, D. G. Alberg, I. Stamenkovic, W. Harmon & R. S. Geha: Cyclosporin A inhibits CD40 ligand expression in T lymphocytes. *J Clin Invest* 93, 1315-1320 (1994)

12 A. Desai-Mehta, L. Lu, R. Ramsey-Goldman & S. K. Datta: Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. *J Clin Invest* 97, 2063-73 (1996)

13 M. Koshy, D. Berger & M. K. Crow: Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. *J Clin Invest* 98, 826-837 (1996)

14 M. R. Kehry: CD40-mediated signaling in B cells. *J Immunol* 156, 2345-2348 (1996)

15 N. Sakata, H. R. Patel, N. Terada, A. Aruffo, G. L. Johnson & E. W. Gelfand: Selective activation of c-Jun kinase mitogen-activated protein kinase by CD40 on human B cells. *J Biol Chem* 270, 30823-8 (1995)

16 I. Berberich, G. Shu, F. Siebelt, J. R. Woodgett, J. M. Kyriakis & E. A. Clark: Cross-linking CD40 on B cells preferentially induces stress-activated protein kinases rather than mitogen-activated protein kinases. *Embo J* 15, 92-101 (1996)

17 S. J. Baker & E. P. Reddy: Transducers of life and death: TNF receptor superfamily and associated proteins. *Oncogene* 12, 1-9 (1996)

18 G. Cheng, A. M. Cleary, Z. S. Ye, D. I. Hong, S. Lederman & D. Baltimore: Involvement of CRAF1, a relative of TRAF, in CD40 signaling. *Science* 267, 1494-1498 (1995)

19 H. M. Hu, K. O'Rourke, M. S. Boguski & V. M. Dixit: A novel RING finger protein interacts with the cytoplasmic domain of CD40. *J Biol Chem* 269, 30069-30072 (1994)

20 G. Mosialos, M. Birkenbach, R. Yalamanchili, T. VanArsdale, C. Ware & E. Kieff: The Epstein-Barr virus transforming protein LMP1 engages signaling proteins for the tumor necrosis factor family. *Cell* 80, 389-399 (1995)

21 T. Sato, S. Irie & J. C. Reed: A novel member of the TRAF family of putative signal transducing proteins binds to the cytoplasmic domain of CD40. *FEBS Letters* 358, 113-118 (1995)

22 R. W. Gedrich, M. C. Gilfillan, C. S. Duckett, J. L. Van Dongen & C. B. Thompson: CD30 contains two binding sites with different specificities for members of the tumor necrosis factor receptor-associated factor family of signal transducing proteins. *J Biol Chem* 271, 12852-12860 (1996)

23 M. Rothe, V. Sarma, V. M. Dixit & D. V. Goeddel: TRAF2-mediated activation of NF-κB by TNF receptor 2 and CD40. *Science* 269, 1424-1427 (1995)

24 T. Morio, S. Hanissian & R. S. Geha: Characterization of a 23-kDa protein associated with CD40. *Proc Natl Acad Sci U S A* 92, 11633-6 (1995)

25 N. Burdin, C. Van Kooten, L. Galibert, J. S. Abrams, J. Wijdenes, J. Banchereau & F. Rousset: Endogenous IL-6 and IL-10 contribute to the differentiation of CD40-activated human B lymphocytes. *J Immunol* 154, 2533-44 (1995)

26 N. Burdin, L. Galibert, P. Garrone, I. Durand, J. Banchereau & F. Rousset: Inability to produce IL-6 is a functional feature of human germinal center B cells. *J Immunol* 156, 4107-4113 (1996)

27 F. Malisan, F. Briere, J. M. Bridon, N. Harindranath, F. C. Mills, E. E. Max, J. Banchereau & H. Martinez-Valdez: Interleukin-10 induces immunoglobulin G isotype switch recombination in human CD40-activated naive B lymphocytes. *J Exp Med* 183, 937-47 (1996)

28 P. Garrone, E. M. Neidhardt, E. Garcia, L. Galibert, C. van Kooten & J. Banchereau: Fas ligation induces apoptosis of CD40-activated human B lymphocytes. *J Exp Med* 182, 1265-73 (1995)

29 E. J. Schattner, K. B. Elkon, D. H. Yoo, J. Tumang, P. H. Krammer, M. K. Crow & S. M. Friedman: CD40 ligation induces Apo-1/Fas expression on human B lymphocytes and facilitates apoptosis through the Apo-1/Fas pathway. *J Exp Med* 182, 1557-65 (1995)

30 S. M. Lens, K. Tesselaar, B. F. den Drijver, M. H. van Oers & R. A. van Lier: A dual role for both CD40-ligand and TNF-alpha in controlling human B cell death. *J Immunol* 156, 507-14 (1996)

31 T. L. Rothstein, J. K. M. Wang, D. J. Panka, L. C. Foote, Z. Wang, B. Stanger, H. Cui, S. T. Ju & A. Marshak-Rothstein: Protection against Fas-dependent Th1-mediated apoptosis by antigen receptor engagement in B cells. *Nature* 374, 163-165 (1995)

32 L. Galibert, N. Burdin, C. Barthelemy, G. Meffre, I. Durand, E. Garcia, P. Garrone, F. Rousset, J. Banchereau & Y. J. Liu: Negative selection of human germinal center B cells by prolonged BCR cross-linking. *J Exp Med* 183, 2075-2085 (1996)

33 C. Lagresle, P. Mondiere, C. Bella, P. H. Krammer & T. Defrance: Concurrent engagement of CD40 and the antigen receptor protects naive and memory human B cells from APO-1/Fas-mediated apoptosis. *J Exp Med* 183, 1377-1388 (1996)

34 L. Galibert, N. Burdin, B. de Saint-Vis, P. Garrone, C. Van Kooten, J. Banchereau & F. Rousset: CD40 and B cell antigen receptor dual triggering of resting B lymphocytes turns on a partial germinal center phenotype. *J Exp Med* 183, 77-85 (1996)

35 K. Wheeler & J. Gordon: Co-ligation of surface IgM and CD40 on naive B lymphocytes generates a blast population with an ambiguous extrafollicular/germinal centre cell phenotype. *Int Immunol* 8, 815-828 (1996)

36 C. Arpin, J. Dechanet, C. Van Kooten, P. Merville, G. Grouard, F. Briere, J. Banchereau & Y. J. Liu: Generation of memory B cells and plasma cells in vitro. *Science* 268, 720-2 (1995)

37 A. Silvy, C. Lagresle, C. Bella & T. Defrance: The differentiation of human memory B cells into specific antibody-secreting cells is CD40 independent. *Eur J Immunol* 26, 517-24 (1996)

38 P. Merville, J. Dechanet, A. Desmouliere, I. Durand, O. de Bouteiller, P. Garrone, J. Banchereau & Y. J. Liu: Bcl-2+ tonsillar plasma cells are rescued from apoptosis by bone marrow fibroblasts. *J Exp Med* 183, 227-36 (1996)

39 W. J. Murphy, S. Funakoshi, M. Beckwith, S. E. Rushing, D. K. Conley, R. J. Armitage, W. C. Fanslow, H. C. Rager, D. D. Taub, F. W. Ruscetti & D. L. Longo: Antibodies to CD40 prevent Epstein-Barr virus-mediated human B cell lymphomagenesis in severe combined immune deficient mice given human peripheral blood lymphocytes. *Blood* 86, 1946-1953 (1995)

40 S. Hess & H. Engelmann: A novel function of CD40: induction of cell death in transformed cells. *J Exp Med* 183, 159-167 (1996)

41 I. S. Grewal, H. G. Foellmer, K. D. Grewal, J. Xu, F. Hardardottir, J. L. Baron, C. A. Janaway & R. A.

Flavell: Requirement for CD40 ligand in costimulation induction, T cell activation, and experimental allergic encephalomyelitis. *Science* 273, 1864-1867 (1996)

42 Y. Yang & J. M. Wilson: CD40 ligand-dependent T cell activation: requirement of B7-CD28 signaling through CD40. *Science* 273, 1862-1864 (1996)

43 M. R. Alderson, R. J. Armitage, T. W. Tough, L. Strockbine, W. C. Fanslow & M. K. Spriggs: CD40 expression by human monocytes: regulation by cytokines and activation of monocytes by the ligand for CD40. *J Exp Med* 178, 669-74 (1993)

44 N. Romani, A. Lenz, H. Glassl, H. Stossel, U. Stanzl, O. Majdic, P. Fritsch & G. Schuler: Cultured human Langerhans cells resemble lymphoid dendritic cells in phenotype and function. *J Invest Dermatol* 93, 600-609 (1989)

45 C. Caux, C. Massacrier, B. Vanbervliet, B. Dubois, C. Van Kooten, I. Durand & J. Banchereau: Activation of human dendritic cells through CD40 cross-linking. *J Exp Med* 180, 1263-72 (1994)

46 L. M. Pinchuk, P. S. Polacino, M. B. Agy, S. J. Klaus & E. A. Clark: The role of CD40 and CD80 accessory cell molecules in dendritic cell-dependent HIV-1 infection. *Immunity* 1, 317-25 (1994)

47 L. J. Zhou & T. F. Tedder: Human blood dendritic cells selectively express CD83, a member of the immunoglobulin superfamily. *J Immunol* 154, 3821-3835 (1995)

48 P. S. Freudenthal & R. M. Steinman: The distinct surface of human blood dendritic cells, as observed after an improved isolation method. *Proc Natl Acad Sci USA* 87, 7698-7702 (1990)

49 J. Peguet-Navarro, C. Dalbiez-Gauthier, F. M. Rattis, C. Van Kooten, J. Banchereau & D. Schmitt: Functional expression of CD40 antigen on human epidermal Langerhans cells. *J Immunol* 155, 4241-7 (1995)

50 K. Gerritse, J. D. Laman, R. J. Noelle, A. Aruffo, J. A. Ledbetter, W. J. A. Boersma & E. Claassen: CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. *Proc Natl Acad Sci USA* 93, 2499-2504 (1996)

51 U. Shu, M. Kiniwa, C. Y. Wu, C. Maliszewski, N. Vezzio, J. Hakimi, M. Gately & G. Delespesse: Activated T cells induce interleukin-12 production by monocytes via CD40-CD40 ligand interaction. *Eur J Immunol* 25, 1125-8 (1995)

52 M. K. Kennedy, K. S. Picha, W. C. Fanslow, K. H. Grabstein, M. R. Alderson, K. N. Clifford, W. A. Chin & K. M. Mohler: CD40/CD40 ligand interactions are required for T cell-dependent production of interleukin-12 by mouse macrophages. *Eur J Immunol* 26, 370-8 (1996)

53 N. Malik, B. W. Greenfield, A. F. Wahl & P. A. Kiener: Activation of human monocytes through CD40 induces matrix metalloproteinases. *J Immunol* 156, 3952-60 (1996)

54 T. Kato, R. Hakamada, H. Yamane & H. Nariuchi: Induction of IL-12 p40 messenger RNA expression and IL-12 production of macrophages via CD40-CD40 ligand interaction. *J Immunol* 156, 3932-8 (1996)

55 E. Stuber, W. Strober & M. Neurath: Blocking the CD40L-CD40 interaction *in vivo* specifically prevents the priming of T helper 1 cells through the inhibition of interleukin 12 secretion. *J Exp Med* 183, 693-8 (1996)

56 F. Koch, U. Stanzl, P. Jennewein, K. Janke, C. Heufler, E. Kampgen, N. Romani & G. Schuler: High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *J Exp Med* 184, 741-746 (1996)

57 M. Cella, D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia & G. Alber: Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J Exp Med* 184, 747-752 (1996)

58 F. Sallusto, M. Cella, C. Danieli & A. Lanzavecchia: Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex Class II compartment: downregulation by cytokines and bacterial products. *J Exp Med* 182, 389-400 (1995)

59 A. D. McLellan, R. V. Sorg, L. A. Williams & D. N. Hart: Human dendritic cells activate T lymphocytes via a CD40: CD40 ligand-dependent pathway. *Eur J Immunol* 26, 1204-10 (1996)

60 P. A. Kiener, P. Moran-Davis, B. M. Rankin, A. F. Wahl, A. Aruffo & D. Hollenbaugh: Stimulation of CD40 with purified soluble gp39 induces proinflammatory responses in human monocytes. *J Immunol* 155, 4917-25 (1995)

61 B. Ludewig, D. Graf, H. R. Gelderblom, Y. Becker, R. A. Kroczek & G. Pauli: Spontaneous apoptosis of dendritic cells is efficiently inhibited by TRAP (CD40-ligand) and TNFa, but strongly enhanced by interleukin-10. *Eur J Immunol* 25, 1943-1950 (1995)

62 L. Flores-Romo, P. Bjorck, V. Duvert, C. Van Kooten, S. Saeland & J. Banchereau: CD40 ligation on human CD34+ hematopoietic progenitors induces their proliferation and differentiation into functional dendritic cells. *J Exp Med* in press, (1996)

63 R. D. Stout, J. Suttles, J. Xu, I. S. Grewal & R. A. Flavell: Impaired T cell-mediated macrophage activation in CD40 ligand-deficient mice. *J Immunol* 156, 8-11 (1996)

64 L. Tian, R. J. Noelle & D. A. Lawrence: Activated T cells enhane nitric oxide production by murine splenic macrophages through gp39 and LFA-1. *Eur J Immunol* 25, 306-309 (1995)

65 J. Pammer, A. Plettenberg, W. Weninger, B. Diller, M. Mildner, A. Uthman, W. Issing, M. Sturzl & E. Tschachler: CD40 antigen is expressed by endothelial cells and tumor cells in Kaposi's sarcoma. *Am J Pathol* 148, 1387-96 (1996)

66 C. Van Kooten, J. S. J. Gerritsma, M. E. Paape, L. A. Van Es, J. Banchereau & M. R. Daha: Possible role for CD40-CD40L in the regulation of interstitial infiltration in the kidney. *Kidney Int* in press (1996)

67 D. Hollenbaugh, N. Mischel-Petty, C. P. Edwards, J. C. Simon, R. W. Denfeld, P. A. Kiener & A. Aruffo: Expression of functional CD40 by vascular endothelial cells. *J Exp Med* 182, 33-40 (1995)

68 M. J. Yellin, J. Brett, D. Baum, A. Matsushima, M. Szabolcs, D. Stern & L. Chess: Functional interactions of T cells with endothelial cells: the role of CD40L-CD40-mediated signals. *J Exp Med* 182, 1857-1864 (1995)

69 K. Karmann, C. C. Hughes, J. Schechner, W. C. Fanslow & J. S. Pober: CD40 on human endothelial cells: inducibility by cytokines and functional regulation of adhesion molecule expression. *Proc Natl Acad Sci U S A* 92, 4342-6 (1995)

70 K. Karmann, C. C. Hughes, W. C. Fanslow & J. S. Pober: Endothelial cells augment the expression of CD40 ligand on newly activated human CD4+ T cells through a CD2/LFA-3 signaling pathway. *Eur J Immunol* 26, 610-7 (1996)

71 R. A. Kroczek, D. Graf, D. Brugnoni, S. Giliani, U. Korthuer, A. Ugazio, G. Senger, H. W. Mages, A. Villa & L. D. Notarangelo: Defective expression of CD40 ligand on T cells causes "X-linked immunodeficiency with hyper-IgM (HIGM1)". [Review]. *Immunol Rev* 138, 39-59 (1994)

72 T. M. Foy, J. D. Laman, J. A. Ledbetter, A. Aruffo, E. laassen & R. J. Noelle: gp39-CD40 interactions are essential for germinal center formation and the development of B cell memory. *J Exp Med* 180, 157-163 (1994)

73 I. S. Grewal, J. Xu & R. A. Flavell: Impairment of antigen-specific T-cell priming in mice lacking CD40 ligand. *Nature* 378, 617-620 (1995)

74 M. Kamanaka, P. Yu, T. Yasui, K. Yoshida, T. Kawabe, T. Horii, T. Kishimoto & H. Kikutani: Protective role of CD40 in Leishmania major infection at two distinct phases of cell-mediated immunity. *Immunity* 4, 275-81 (1996)

75 K. A. Campbell, P. J. Ovendale, M. K. Kennedy, W. C. Fanslow, S. G. Reed & C. R. Maliszewski: CD40 ligand is required for protective cell-mediated immunity to Leishmania major. *Immunity* 4, 283-289 (1996)

76 L. Soong, J. C. Xu, I. S. Grewal, P. Kima, J. Sun, B. Longley Jr., N. H. Ruddle, D. McMahon-Pratt & R. A. Flavell: Disruption of CD40-CD40 ligand interactions results in an enhanced susceptibility to Leishmania amazonensis infection. *Immunity* 4, 263-73 (1996)

77 I. C. M. MacLennan: Germinal centers. *Ann Rev Immunol* 12, 117-139 (1994)

78 F. Facchetti, C. Appiani, L. Salvi, J. Levy & L. D. Notorangelo: Immunohistologic analysis of ineffective CD40-CD40 ligand interaction in lymphoid tissues from patients with X-linked immunodeficiency with hyper-IgM. Abortive germinal center cell reaction and severe depletion of follicular dendritic cells. *J Immunol* 154, 6624-6633 (1995)

79 Y. W. Chu, E. Marin, R. Fuleihan, N. Ramesh, F. S. Rosen, R. S. Geha & R. A. Insel: Somatic mutation of human immunoglobulin V genes in the X-linked hyper IgM syndrome. *J Clin Invest* 95, 1389-1393 (1995)

80 D. Razanajaona, C. Van Kooten, S. Lebecque, J. M. Bridon, S. Ho, S. Smith, R. Callard, J. Banchereau & F. Briere: Somatic mutations in human Ig variable genes correlate with a partially functional CD40-ligand in the X-linked hyper-IgM syndrome. *J Immunol* 157, 1492-1498 (1996)

81 L. Galibert, J. Van Dooren, I. Durand, F. Rousset, R. Jefferis, J. Banchereau & S. Lebecque: Anti-CD40 plus interleukin-4-activated human naive B cell lines express unmutated immunoglobulin genes with intraclonal heavy hain isotype variability. *Eur J Immunol* 25, 733-737 (1995)

82 E. Kallberg, S. Jainandunsing, D. Gray & T. Leanderson: Somatic mutation of immunoglobulin V genes in vitro. *Science* 271, 1285-9 (1996)

83 D. Van Essen, H. Kikutani & D. Gray: CD40 ligand-transduced co-stimulation of T cells in the development of helper function. *Nature* 378, 620-623 (1995)

84 M. H. Blotta, J. D. Marshall, R. H. DeKruyff & D. T. Umetsu: Cross-linking of the CD40 ligand on human CD4+ T lymphocytes generates a costimulatory signal that up-regulates IL-4 synthesis. *J Immunol* 156, 3133-40 (1996)

85 X. Peng, A. Kasran, P. A. M. Warmerdam, M. De Boer & J. L. Ceuppens: Accessory signaling by CD40 for T cell activation: induction of Th1 and Th2 cytokines and synergy with interleukin-12 for interferon-gamma production. *Eur J Immunol* 26, 1621-1627 (1996)

86 F. H. Durie, A. Aruffo, J. Ledbetter, K. M. Crassi, W. R. Green, L. D. Fast & R. J. Noelle: Antibody to the ligand of CD40, gp39, blocks the occurrence of the acute and chronic forms of graft-vs-host disease. *J Clin Invest* 94, 1333-8 (1994)

87 J. E. Buhlmann, T. M. Foy, A. Aruffo, K. M. Crassi, J. A. Ledbetter, W. R. Green, J. C. Xu, L. D. Shultz, D. Roopesian, R. A. Flavell, L. Fast, R. J. Noelle & F. H. Durie: In the absence of a CD40 signal, B cells are tolerogenic. *Immunity* 2, 645-53 (1995)

88 D. C. Parker, D. L. Greiner, N. E. Phillips, M. C. Appel, A. W. Steele, F. H. Durie, R. J. Noelle, J. P. Mordes & A. A. Rossini: Survival of mouse pancreatic islet allografts in recipients treated with allogeneic small lymphocytes and antibody to CD40 ligand. *Proc Natl Acad Sci U S A* 92, 9560-4 (1995)

89 C. P. Larsen, D. Z. Alexander, D. Hollenbaugh, E. T. Elwood, S. C. Ritchie, A. Aruffo, R. Hendrix & T. C. Pearson: CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation* 61, 4-9 (1996)

90 C. P. Larsen, E. T. Elwood, D. Z. Alexander, S. C. Ritchie, R. Hendrix, C. Tucker-Burden, H. R. Cho, A. Aruffo, D. Hollenbaugh, P. S. Linsley, K. J. Winn & T. C. Pearson: Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature* 381, 434-438 (1996)