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(54) Title: HUMAN RECEPTOR H4-1BB

(57) Abrégé/Abstract:
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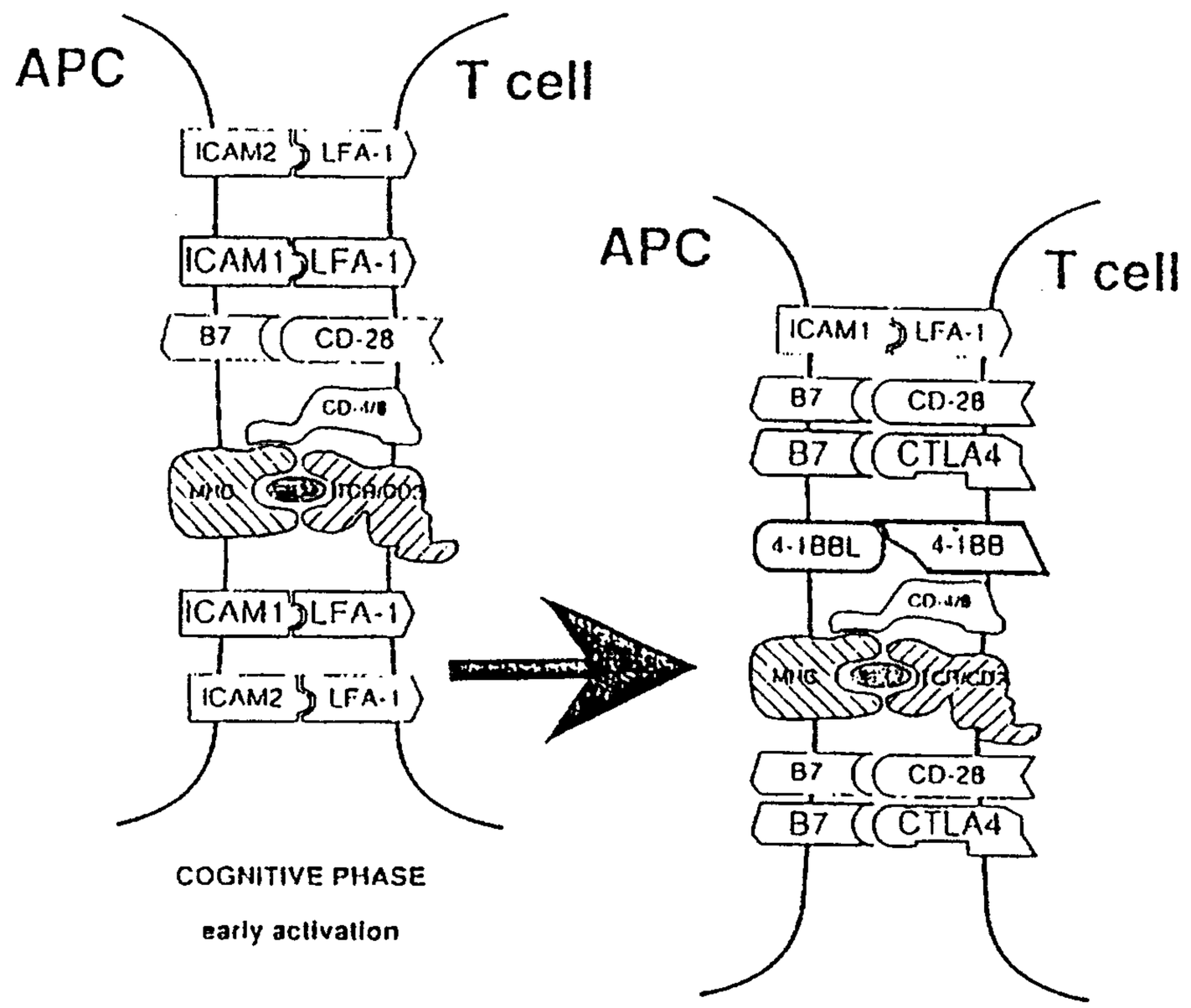
(57) Abstract

The human receptor H4-1BB has been isolated, sequenced and disclosed herein. The cDNA of the human receptor H4-1BB is about 65 % homologous to the mouse cDNA 4-1BB and was isolated by using probes derived from cDNA 4-1BB. A fusion protein for detecting cell membrane ligands to human receptor protein H4-1BB was developed. It comprises the extracellular portion of the receptor protein H4-1BB and a detection protein (alkaline phosphatase) bound to the portion of the receptor protein H4-1BB. B-cells that have expressed a ligand to receptor protein H4-1BB can be treated with cells that have expressed receptor protein H4-1BB and B-cell proliferation may be induced. The use of H4-1BB to block H4-1BB ligand binding has practical application in the suppression of the immune system during organ transplantation. A monoclonal antibody against H4-1BB can be used to enhance T-cell proliferation by treating T-cells that have expressed receptor protein H4-1BB with the anti H4-1BB monoclonal antibody. Tumors transfected with H4-1BBL may be capable of delivering antigen-specific signals as well as the co-stimulatory signals and can be killed by human cytotoxic T lymphocytes.



HUMAN RECEPTOR H4-1BB

FIELD OF THE PRESENT INVENTION

The present invention relates to a previously unknown human receptor protein, H4-1BB, which was isolated and identified based upon work with a homologous murine (mouse) receptor protein, 4-1BB, which was isolated and identified by specific expression of the T cell genes by the present inventor.

BACKGROUND OF THE PRESENT INVENTION

The immune system of humans and other species requires that white blood cells be made in the bone marrow, which white blood cells include phagocytes, lymphocytes and B cells. As presently understood, the phagocytes include macrophage cells which scavenge unwanted materials such as

virus protein from the system. The lymphocytes include helper T cells and killer T cells and B cells as well as other cells, including those categorized as suppressor T cells. The B cells produce the antibodies. The killer T cells physically pierce the cell and the helper T cells facilitate the whole process. In any event, the immune process is facilitated by lymphokines.

Lymphokines are the proteins by which the immune cells communicate with each other. Scientists produce them in sufficient quantities for therapeutic use against immunologic diseases. There are many known lymphokine proteins and they include the interferons, interleukin-1,2,3,4,5,6,7, colony-stimulating factors, lymphotoxin, tumor necrosis factor and erythropoietin, as well as others.

Interleukin 1, secreted from macrophages activate the helper T cells and raise the body temperature causing fever which enhances the activity of the immune cells. The activated helper T Cells produce Interleukin 2 and Interleukin 2 stimulates the helper and killer T cells to grow and divide. The helper T cells also produce another lymphokine, B cell growth factor (BCGF), which causes B cells to multiply. As the number of B cells increases, the helper T cells produce another lymphokine known as the B cell differentiating factor (BCDF), which instructs some of the B cells to stop replicating and start producing antibodies. T cells also produce a lymphokine, gamma interferon (IF), which has multiple effects like Interleukin 2. Interferon helps activate killer T cells, enabling them to attack the invading organisms. Like BCGF, interferon increases the ability of the B cells to produce antibodies. Interferon also affects the macrophages to keep them at the site of the infection and help the macrophages to digest the cells they have engulfed. Gathering momentum with each kind of lymphokine signal between the macrophages and the T cells, the lymphokines amplify the immune system response and the virus protein or

other foreign matter on the infected cells is overwhelmed. There are many other lymphokines, maybe a hundred or more, which participate in the immune process. Many lymphokines are known and many are not.

Lymphokines are sometimes called intercellular peptide signals. Among scientists there is widespread use of cloned cell lines as lymphokine producers and the isolation of lymphokine mRNA has become a common technique. The mouse receptor protein, 4-1BB, was isolated and identified based on specific expression of the T cell genes using a technique identified by the present inventor in a publication (Proc. Natl. Acad. Sci. USA. 84, 2896-2900, May 1987, Immunology). The protocol reported in this publication can be used by scientists to detect virtually all of the lymphokines. The method is designed to detect virtually all mRNA expressed differentially and the mRNA sequences of the immune cells are expressed differentially (as they relate to the T cells and the killer T cells) even though the level of expression is low and the quantity of the secreted lymphokine protein is low. The present inventor believes that the analysis described in the above identified publication can reveal biologically important molecules such as lymphokines because there are many indications that biologically important or active molecules are coded by the most scarce messages. An example is a transforming growth factor (TGF) which is present as only one of a million clones.

Most T cell factors have been classically identified by recognizing biologic activities in assays, purifying the protein information. An alternative approach is to isolate putative T cell genes based upon specific expression and then demonstrate the function of the unknown molecule. Using the aforesaid modified differential screening procedure, the present inventor cloned a series of T cell subset-specific cDNAs from cloned helper T (HTL) L2 and cloned cytolytic T lymphocyte (CTL) L3.

A series of T-cell subset-specific cDNAs were isolated from cloned murine T-cells by employing a modified differential screening procedure. The nucleotide sequence and expression properties of some of the cDNA species have been reported. One of the genes not previously characterized, that encodes mouse receptor protein 4-1BB, was studied further. These studies have led to the isolation of the human homologue to 4-1BB, H4-1BB.

SUMMARY OF THE PRESENT INVENTION

The present invention includes the human receptor protein H4-1BB and the cDNA gene encoding for human receptor protein H4-1BB. The nucleotide sequence of the isolated cDNA is disclosed herein along with the deduced amino acid sequence. The cDNA gene identified as pH4-1BB was deposited at the Agricultural Research Service Culture Collection and assigned the accession number: NRRL B21131

The cDNA, and fragments and derivatives thereof, can be used as a probe to isolate DNA sequences encoding for proteins similar to the receptor protein encoded by the cDNA. The cDNA of the human receptor H4-1BB is about 65% homologous to the mouse cDNA 4-1BB and was isolated by using probes derived from cDNA 4-1BB. The cDNA gene identified as p4-1BB was deposited at the American Type Culture Collection at 12301 Parklawn Drive, Rockville, Maryland 20852 under ATCC No.: 67825.

The human receptor protein H4-1BB can be produced by: 1) inserting the cDNA of H4-1BB into an appropriate expression vector, 2) transfecting the expression vector into an appropriate transfection host, c) growing the transfected hosts in appropriate culture media and d) purifying the receptor protein from the culture media. The protein and fragments and derivatives can be used: 1) as a probe to isolate ligands to human receptor protein H4-

1BB, 2) to stimulate proliferation of B-cells expressing H4-1BB ligands, or 3) to block H4-1BB ligand binding.

B-cell proliferation can be induced by treating B-cells that have expressed a ligand to receptor protein H4-1BB with cells that have expressed receptor protein H4-1BB. The use of H4-1BB to block H4-1BB ligand binding has practical application in the suppression of the immune system during organ transplantation. A similar costimulatory immune system pathway is being analyzed for this type of application. See "Mounting a Targeted Strike on Unwanted Immune Responses", Jon Cohen, Science, Vol. 257, 8-7-92; "Long Term Survival of Xenogeneic Pancreatic Islet Grafts Induced by CTLA4Ig", Lenschow et al, Science Vol. 257, 7-8-92; and "Immunosuppresion in Vivo by a Soluble Form of the CTLA-4 T Cell Activation Molecule", Linsley et al, Science Vol. 257 7-8-92.

A monoclonal antibody against H4-1BB can be used to enhance T-cell proliferation by treating T-cells that have expressed receptor protein H4-1BB with the anti H4-1BB monoclonal antibody. Some tumors are potentially immunogenic but do not stimulate an effective anti-immune response in vivo. Tumors may be capable of delivering antigen-specific signals to T cells, but may not deliver the co-stimulatory signals necessary for full activation of T cells. Expression of the co-stimulatory ligand B7 on of melanoma cells was found to induce the rejection of a murine melanoma in vivo. ("Tumor Rejection After Direct Co-Stimulation of $CD8^+$ T Cells by B7-Transfected Melanoma Cells", Sarah E. Townsend and James P. Allison, Science Vol. 259, 1-5-93.) A monoclonal antibody against H4-1BB may be capable of the same effect as it is now known to enduce T cell proliferation and activation.

A fusion protein for detecting cell membrane ligands to human receptor protein H4-1BB was developed. It comprises the extracellular portion of the receptor protein H4-1BB and a detection protein (alkaline phosphatase) bound to the portion of the receptor protein H4-1BB. The portion

of the receptor protein H4-1BB binds to the cell membrane ligands and binding can be detected by relative activity assays for the detection protein. The fusion protein is placed in the presence of a cell suspected to express the receptor protein H4-1BB. Then the cell is washed of any fusion protein not bound to the cell membrane ligands. Once the washed cells are placed in the presence of a substrate for the detection protein and the relative activity of the detection protein can be measured.

The primary object of the present invention is the identification of the new human receptor, H4-1BB as identified herein by its sequence.

Another object of the present invention is to teach a fusion protein comprising the extracellular portion of H4-1BB and a detection protein.

Still another object of the present invention is to teach methods of using the cDNA H4-1BB, the receptor protein H4-1BB, the monoclonal antibody and the ligand for H4-1BB.

BRIEF DESCRIPTIONS OF THE FIGURES

Figures 1A and 1B show the sequence for the cDNA of mouse receptor protein 4-1BB and the regions used as PCR primers to obtain the human homologue H4-1BB.

Figures 2a and 2b show the nucleotide sequence and the deduced amino acid sequence of human receptor H4-1BB respectively.

Figures 3a and 3b illustrate the molecules involved in T-cell activation.

Figures 4a, 4b, and 4c illustrate a normal T-cell activation pathway.

Figures 5a, 5b, and 5c illustrate CTLA4-1g alone, 4-1BB/AP and CTLA4-1g together and 4-1BB/AP alone respectively being used to block steps in the T-cell activation pathway.

DETAILED DESCRIPTION

In the following detailed description references are made to known procedures and studies, as well as published work of the applicant. These publications are listed in an appendix included at the end of this detailed description.

Isolation and characterization of mouse receptor 4-1BB

Figures 1A and 1B show the nucleotide sequence and the deduced amino acid sequence of the mouse receptor 4-1BB. The nucleotides of the message strand are numbered in the 5' to 3' direction and numbers are shown on both sides of the sequence. **Nucleotide residue 1 is the A of the initiation codon ATG, and the nucleotides on the 5' side of residue 1 are indicated by negative numbers. The predicted amino acid sequence is shown below the nucleotide sequence. Putative signal peptide is underlined. Stop codon is indicated by (---). Cysteine residues are highlighted by the dots. An unusual feature of 4-1BB sequence is that there is a potential polyadenylation signal of AATAAA at** nucleotides 1158-1163 (Fig. 1B boxed). It was believed that **this signal was functional because this gene produces at least two different sizes of mRNA.**

The transcript of 4-1BB was inducible by concanavalin A in mouse splenocytes, T-cell clones, and hybridomas. The expression of 4-1BB transcripts was inhibited by cyclosporin A. The 4-1BB mRNA was inducible by antigen receptor stimulation but was not inducible by Il-2 stimulation in the cloned T-cells (1). The 4-1BB cDNA encodes a peptide of 256 amino acids containing a putative leader sequence, a potential membrane anchor segment, and other features of known receptor proteins. Therefore, the expression pattern of 4-1BB resembles those of lymphokine mRNAs while the sequence appeared consistent with those of receptor proteins.

The major species of 4-1BB on the cell surface appears to be a 55-kDa dimer. 4-1BB also appears to exist as a 30-kDa monomer and possibly as a 110-kDa tetramer. Since these 4-1BB species were immunoprecipitated from a homogenous population of cells (T cell clone F1), all forms potentially co-exist on each cell. A comparison of peptide digests from the 4-1BB monomer and dimer will be needed to determine whether 4-1BB exists as a homodimer on the cell surface. A variety of cell surface receptors such as the insulin receptor (2), the B cell surface immunoglobulin receptor (3), the T cell Ag receptor (4), the CD28 costimulatory receptor (5), and the CD27 T cell antigen (6) are composed of disulfide-bonded subunits. Receptor dimerization may be required for ligand binding and subsequent biochemical signaling.

4-1BB is not expressed on resting T cells but is inducible by activators which deliver a complete growth stimulus to the T cell. The combination of PMA and ionomycin is capable of mimicing those signals required for T cell proliferation. Although PMA or ionomycin alone induced 4-1BB mRNA, the combination of PMA and ionomycin resulted in optimal 4-1BB expression. Furthermore, the expression of 4-1BB was not transient. When purified splenic T cells were stimulated with immobilized anti-CD3, 4-1BB mRNA was expressed and this expression was maintained for up to 96 hrs poststimulation. Cell cycle analysis will be required to confirm that 4-1BB is expressed throughout cell cycle progression.

4-1BB is structurally related to members of the nerve growth factor receptor super-family. Although these receptors possess structurally similar ligand-binding properties (cysteine-rich regions), the cytoplasmic domains of these proteins are nonconserved which could allow for diversity in transmembrane signaling. Some members of this family are involved in the T or B cell activation process. There are in vitro functional data on the OX-40, CD40 and CD27 antigens. Antibodies against the OX-40 augment the T

cell response in a mixed lymphocyte reaction (7) and antibodies against CD40 enhance B-cell proliferation in the presence of a coactivator, such as PMA or CD20 antibodies, and synergize with IL-4 in vitro to induce B-cell differentiation and to generate long-term normal B cell lines (8). One monoclonal antibody, anti-1A4, which recognizes an epitope on the CD27 molecule inhibited calcium mobilization, IL-2 secretion, helper T cell function, and T cell proliferation. On the other hand, CLB-CD27/1, another anti-CD27 mAb enhanced proliferation of hunan T cells stimulated with PHA or anti-CD3 mAb (6). These results indicate that the CD27 molecule plays an important role in T cell activation. Except for TNFRs, NCFR and CD40, the ligands or cell surface molecules to which the members of the superfamily bind are not yet identified. Identification and characterization of the ligands to which the receptors bind will be helpful in better defining the physiologic role of 4-1BB.

To ascertain whether cell surface 4-1BB could contribute to T cell activation, the anti-4-1BB 53A2 was used as an antagonist to 4-1BB. These data suggested that 4-1BB does in fact have the potential to function as an accessory signaling molecule during T cell activation and proliferation. The addition of soluble 53A2 to purified splenic T cells stimulated with immobilized anti-CD3 resulted in an amplification of ^{3}H thymidine incorporation compared to T cells stimulated with anti-CD3 alone. This pattern of enhancement ranged from 2- to 10- fold in three independent experiments.

In the original two signal model of Bretcher and Cohn, they proposed that signal 1 , the occupancy of the T cell antigen receptor (TCR), resulted in inactivation of the T cell in the absence of signal 2, which is provided by accessory cells. This has since been confirmed by a variety of studies (9). The identification of the accessory cell CD28 as a potent costimulatory receptor on T cells was a significant contribution in beginning to charactize the

accessory signal(s) required for optimal T cell proliferation **(10)**. It is possible that other cell surface molecules may contribute to these costimulatory activation requirements **(11)**.

The biochemical signals delivered through 4-1BB are not completely known. One possibility considered was the observation that 4-1BB contains a putative $p56^{lck}$ tyrosine kinase binding domain in its cytoplasmic tail. It was later determined that $p56^{lck}$ tyrosinase kinase binds to 4-1BB. It will also be worthwhile to determine if 4-1BB-mediated signaling can regulate genes such as IL-2 and IL-2 receptor, whose expression is required for T cell activation and subsequent proliferation.

Although the precise functions of members of the Nerve Growth Factor Receptor (NGFR) family appear to be diverse, an emerging theme is one in which these molecules may contribute in various ways to a maintenance of responsiveness or viability of the particular cell type in which they are expressed. For instance, NGF is absolutely required for viability of neurons in vitro and in vivo **(12)**. The crosslinking of CD40 by soluble antiCD40 monoclonal antibody blocks germinal center centrocytes from undergoing apoptosis in vitro **(13)**. Signals delivered throug CD40 may also aid in maintenance of responsiveness to differentiation factors. The ligation of CD40 with anti-CD40 $F(ab')_2$ fragments in the presence of IL-4 induced large increases IgE synthesis **(14)**. Also, anti-CD40 activated naive B cells treated with IL-10 and transforming growth factor-β became committed to IgA secretion **(15)**.

In addition to sharing the molecular characteristics with the NGFR superfamily, it was noted that the 4-1BB contained a putative zinc finger structure of the yeast eIF-2β protein **(16)**. 4-1BB also shares a conserved region with the *sina seven in absentia of Drosophila*, which is required for correct photoreceptor cell development **(17)**. That particular region is also similar to the protein

product of the DG17 gene of *Dictyostelium*, whose expression is specificaily induced during aggregation by cAMP **(18)**.

This region forms the pattern of $C-X_2-C-X_9-C-X_3-H-X_3-C-X-C$; and the cysteines and histidine are conserved in a similar space in 4-1BB, *sina*, and DG17 proteins. Ten of 24 amino acids between the 4-1BB and *sina* proteins are identical, and 3 of 24, are conservative substitutes. The conserved pattern suggests that these amino acids are functionally important. The *sina* protein is localized in the nucleus, suggesting that it has a regulatory function in cells. The fact that the amino acid sequence of 4-1BB contains features like a zinc finger motif, a nuclear protein, and a receptor domain suggests that 4-1BB may play diverse roles during cellular proliferation and differentiation.

4-1BB may represent another cell-surface molecule involved in T cell-APC interactions. The 4-1BB-AP fusion protein specifically bound to mature B-cell lines, anti-μ-activated primary B cells, and mature macrophage-cell lines. 4-1BB-AP bound at low or insignificant levels to immature B- and macrophage-cell lines, T-cell clones, T-cell lines, primary culture T cells, and various nonlymphoid-cell lines. Since 4-1BB-AP binds to mature B cells and macrophages, it is possible that signals delivered upon 4-1BB binding may modulate APC functions in some way. This possibility remains to be explored.

Chalupny and colleagues **(19)** have proposed that 4-1BB Rg, a fusion protein consisting of the extracellular domain of 4-1BB and the Fc region of human IgG, bound to the extracellular matrix (ECM). The highest level of 4-1BB Rg binding was to human vitronectin. In data not shown, an ELISA was performed using 4-1BB-AP and human vitronectin (Yelios Pharmaceuticals/GIBCO-BRL, Grand Island, NY.) immobilized at 0.007 μg-10 μg per well on microtiter plates. No binding of 4-1BB-AP based on AP activity was observed. To rule out the possibility that 4-1BB-AP was binding to proteins extrinsically attached to the cell

surface (possible extracellular matrix components), B-cell lymphomas were washed in acid conditions prior to the binding assay. 4-1BB-AP still bound specifically to mature B-cell lymphomas. It is still to be determined whether a 4-1BB-ligand specifically expressed on B cells and macrophages exists, and whether 4-1BB-AP may bind to the ECM under particular binding conditions. It is possible that the ECM could facilitate the binding of 4-1BB to a specific cell-surface ligand.

B cells and helper T cells interact with each other through receptors on B cells binding to their specific counter-receptors on T cells. It is thought that this interaction results in a cascade of biochemical signaling relays between these two cell types **(20)**. As this interaction proceeds, these cells become committed to enter the S phase of the cell cycle. Initial interactions between TCR and CD4 on T cells, and processed antigen-MHC II on B cells, do not result in B cells capable of entering the cell cycle **(21)**. However, studies from in vitro systems suggest that once T-cells are stimulated, they express newly synthesized or modified cell-surface molecules capable of inducing B cells to enter the cell cycle **(22, 23)**. This T-cell function is not antigen-specific or MHC-restricted **(24)**. In addition, soluble factors are not required for the activated Th induction of B-cell activation **(25)**. Once B cells enter the cell cycle, IL-4 induces B cells to progress from G_1 to S phase. The ability of activated T cells or T-cell membranes to promote the entry of B cells into the cell cycle can be blocked by either cycloheximide or cyclosporin A treatment **(26, 27)**. These newly expressed membrane proteins appear to be "lymphokine-like" in their induction characteristics.

4-1BB has expression properties which meet the requirements of a B-cell costimulator. 4-1BB is inducible by anti-CD3 or TCR-mediated T-cell stimulation, and its expression is sensitive to cyclosporin A as well as cycloheximide treatment **(28)**. Interestingly,

paraformaldehyde-fixed SF21-4-1BB cells, synergized with anti-μ in inducing B-cell proliferation. The costimulation of splenic B cells by SF21-4-1BB occurred at optimal (10 μg/ml) and suboptimal (1.0-0.1 μg/ml) doses of anti-μ. The addition of SF21-4-1BB cells to resting B cells, did not result in significant B-cell proliferation. SF21-4-1BB cells did not synergize with TPA or ionomycin, or suboptimal concentrations of LPS in inducing B-cell proliferation.

Although the baculovirus system has been used to express large amounts of recombinant soluble proteins, this system may be utilized for the expression of recombinant cell-surface proteins. The baculovirus infection provides a convenient means to express uniformity high levels of recombinant protein on a per cell basis. It is noteworthy, that the addition of SF21 cells alone did not result in significant levels of costimulation. This can be a potential problem when using cos- or L- cell lines which can exhibit strong costimulator activity on their own.

Another member of the NGFR superfamily, CD40, is expressed on B cells and interacts with gp39, a molecule expressed on activated T cells. The cDNAs encoding the murine (29) and human (30) gp39 proteins have been cloned; this cell surface molecule is a type II membrane protein with homology to tumor necrosis factor. Noelle et al. (31) found that a CD40-immunoglobulin fusion protein, is capable of blocking T cell-induced B-cell proliferation and differentiation in a dose-dependent manner. Armitage et al. have isolated a cDNA for murine gp39 and showed that gp39 could induce B-cell proliferation in the absence of co-stimuli, and result in IgE production in the presence of IL-4-. Hollenbaugh et al. (32) have shown that COS cells transfected with human gp 39 can synergize with either TPA or anti-CD20 in inducing human B-cell proliferation and is able to stimulate B cells without a costimulator only at low levels. These data indicate that CD40 may be one of the

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B-cell-surface molecules that transmit signals during physical contact with T cells.

Cell-surface receptors communicate with their external milieu by interacting either with soluble factors or other cell surface molecules expressed on neighboring cells. The role of biochemical signals delivered by cell-cell contact versus those delivered by soluble factors interacting with cell surface receptors is not clear. The NGFR superfamily is unusual for the TNFR I and II as well as the NGFR bind to more than one ligand. The TNFRs I and II both bind to TNF-α and TNF-R (33). The NGFR binds to NGF, brain-derived neurotrophic factor, and neurotrophin-3 (34).

In addition, one ligand may function as both a cell surface and soluble ligand. Recent evidence on the CD40 ligand, gp39, suggests that this ligand can exist as a membrane bound as well as a soluble ligand (35). It may be possible that 4-1BB is secreted and interacts with B cells in a soluble form as well as a membrane bound form. A member of the NGFR receptor family, CD27, which is expressed on T cells, is secreted in addition to being expressed on the cell surface (36). It is also possible that more than one ligand (soluble and cell surface) may bind to 4-1BB.

Isolation of the human homologue, H4-1BB

In order to isolate the human homologue (H4-1BB) of mouse 4-1BB two sets of polymerase chain reaction (PCR) primers were designed. To design the PCR primers, the amino acid sequence among the members of nerve growth factor receptor (NGFR) superfamily were compared because 4-1BB is a member of the superfamily (37). The amino acid sequences employed were mouse 4-1BB (38), human NGFR (39), human tumor necrosis factor receptors (33), human CD40 (40), and human CD27 (6). The areas of sequence conservation among the NGFR superfamily were chosen.

Forward primer I (H4-1BBFI) spans from amino acids 36 to 41 and forward primer II (HR-1BBFII) spans from amino

acids 52 to 58 of the mouse 4-1BB. Reverse primer I (H4-1BBRI) spans from amino acids 116 to 121 and reverse primer II (H4-1BBRII) spans from amino acids 122 to 128 of mouse 4-1BB. The regions used as PCR primers in mouse 4-1BB are indicated if Fig. 1.

The degenerate oligonucleotide sequence of each primer is as follows:

H4-1BBFI:	5′ TTC TGT CGI AAA TAT AAT CC 3′	
	T C A G C C	
H4-1BBFII:	5′ TTC TCI TCI ATT GGI GGI CA 3′	
	T G G C	
	A	
H4-1BBRI:	5′ CC IAA IGA ACA IGT TTT ACA 3′	
	G CT G C G	
H4-1BBRII:	5′ TT TTG ATC ATT AAA IGT ICC 3′	
	C G G G	

Peripheral blood lymphocytes from normal healthy individuals were isolated and activated with PMA (10 ng/ml) and ionomycin (1 μM). mRNA from the lymphocytes was isolated. Using reverse transcriptase the human lymphocyte mRNA was converted to single-stranded cDNA. The cDNA was then amplified with Taq polymerase with combination of the primers. The combination of primers was as follows: H4-1BBFI vs H4-1BBRI; H4-1BBFI vs H4-1BBRII; H4-1BBFII vs H4-1BBRI; and H4-1BBFII vs H4-1BBRII.

The primer set of H4-1BBFII and H4-1BBRII produced a specific band of ~240bp. The 240bp is an expected size of human 4-1BB if the human homologue protein is similar to mouse 4-1BB in size. The PCR product (240bp) was cloned in PGEM3 vector and sequenced. One open reading frame of the PCR product was ~65% identical to mouse 4-1BB. Therefore, it was concluded that the 240 bp PCR product is the human homologue of mouse 4-1BB. The 240 bp PCR product was used to screen λgt11 cDNA library of activated human T lymphocytes. An ~0.85 kb cDNA was isolated. The sequence of the cDNA is shown in Figure 2 and the predicted amino acid sequence is shown in Figure 2b. The same information

is shown is the sequence listing attached to this specification in SEQ. ID. NO. 1.

An expression plasmid to produce H4-1BB-AP fusion protein was constructed. The 5′ portion of the H4-1BB cDNA including sequences encoding the signal sequence and the entire extracellular domain, was amplified by PCR. For correctly oriented cloning, a Hind III site on the 5′ end of the forward primer and a Bg1 II site on the 5′ end of the reverse primer were created.

The Hind III - Bg1 II H4-1BB fragment was inserted into the mammalian expression vector APtaq-1, upstream of the coding sequence for human placental alkaline phosphatase (AP). The oligonucleotides PCR primers used for the amplification of 5′ portion of H4-1BB are as follows:

Forward primer: 5′ AAT AAG CTT TGC TAG TAT CAT ACC T 3′

Reverse primer: 5′ TTA AGA TCT CTG CGG AGA GTG TCC TGG CTC 3′

H4-1BB-AP will be used to identify cells and tissues that express ligand for human 4-1BB (i.e. H4-1BBL). The studies with mouse 4-1BB indicated that the ligand for 4-1BB is on the cell suface. B cells and macrophages were major cells that express 4-1BBL. It is expected that H4-1BBL also expresses on human B cells and macrophages.

A mammalian expression cDNA library will be generated from human cell lines that express H4-1BBL. The library will be screened by [^{125}] I-labeled H4-1BB-AP. cDNA for H4-1BBL will then be isolated and characterized. Soluble recombinant H4-1BBL will then be produced. Both H4-1BB-AP and H4-1BBL will be used to suppress or enhance immune responses as described below. Monoclonal antibody to H4-1BB and H4-1BBL will be produced.

According to studies with mouse 4-1BB, 4-1BB acts as a costimulatory signal. It is expected that H4-1BB will

act as a costimulatory signal for T cell activation. Mouse 4-1BB helped B cells with proliferation and differentiation. It is expected that H4-1BB will do the same. H4-1BB-AP, H4-1BBL and monoclonal antibody can be used to suppress or enhance human immune responses.

Figures 3a and 3b illustrate the molecules involved in T-cell activation. During early T-cell activation (cognitive phase), resting T cells express the TCR/CD3 complex and other "accessory" molecules. Among these constitutively expressed molecules, CD4 (or CD8), LFA-1 and CD28 are probably the ones to receive costimulatory signals. Initial interaction with the TCR/CD3 complex in combination with these 'accessory' costimulatory signals leads to subsequent expression of additional receptor molecules such as CD28, CTLA4, and 4-1BB. These newly expressed molecules are probably going to receive additional important costimulatory signals at later stages of T-cell activation (clonal expansion).

<u>Suppression of immune responses.</u>

Figures 4a-c illustrate a normal T-cell acivation pathway. Figures 5a-c illustrate the blocking of immune responses with soluble chimera of 4-1BB. If 4-1BB plays a role in T-cell activation, blocking of the interaction to its ligand on antigen-presenting cells should result in suppression of T-cell dependent immune responses. It is well documented that blocking of the interaction of CD28 to its counter-receptor B7 suppresses in varying degrees, both in vivo antibody production and cell-mediated immune responses. Blocking of both interactions should result in a more effective immunosuppression; since 4-1BB is induced during T-cell activation. Blocking of the interaction of 4-1BB to its ligand may be of importance at later stages of the activation process where the CD28/B7 interaction may no longer be of relevance.

As illustrated with mouse receptor 4-1BB and mouse ligand 4-1BBL above, addition of H4-1BB-AP will coat the H4-

C

1BBL expressing cells and block the normal interaction between H4-1BB and H4-1BBL. This will lead to immunosuppression. This type of immunosuppression is antigen-specific. Therefore it avoids the generalized immunosuppression produced by antiCD3 or cyclosporin A treatments. H4-1BB-AP treatment can be used to treat certain autoimmune diseases and to facilitate organ transplantation.

Immune enhancement.

H4-1BB may function at the late stage of T cell activation and may be a critical molecule for completion of T cell activation. Most tumors display tumor-specific antigens. One reason, however, why immunogenic tumors can escape host immunity is that tumor-reactive T cells receive inadequate costimulation. The introduction of the costimulatory molecules, such as H4-1BB into the tumor, therefore, could enhance the antitumor immunity of cytotoxic T cells (CTL). H4-1BBL can be expressed in cell-specific fashion. For example, the H4-1BBL can be expressed in melanoma using melanocyte-specific promoter such as tyrosinase promoters. The H4-1BBL-expressing melanoma will stimulate cytotoxic T cells through H4-1BB and activate the melanoma-specific CTL. The activated melanoma-specific CTL can destroy melanoma.

Appendix to References

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The foregoing description has been directed to particular embodiments of the invention in accordance with the requirements of the Patent Statutes for the purposes of illustration and explanation. It will be apparent, however, to those skilled in this art that many modifications and changes will be possible without departure from the scope and spirit of the invention. It is intended that the following claims be interpreted to embrace all such modifications.

SEQUENCE LISTING

(1) GENERAL INFORMATION:
(i) APPLICANT: Byoung Se Kwon
(ii) TITLE OF INVENTION: New Human Receptor and Related Products and Methods
(iii) NUMBER OF SEQUENCES: 1
(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Barnard & Brown
(B) STREET: 306 E. State St., Suite 220
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 3.50 inch, 1.4Mb storage
(B) COMPUTER: IBM AT Compatible
(C) OPERATING SYSTEM: MS DOS, Version 5.0
(D) SOFTWARE: Special QBasic program
(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/012,269
(B) FILING DATE: 2/1/93
(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/922,996
(B) FILING DATE: 7/30/92
(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/267,577
(B) FILING DATE: 11/7/88
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(C) TELEX:
(2) INFORMATION FOR SEQ ID NO: 1 :
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 838
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double stranded
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA to mRNA
(iii) HYPOTHETICAL: No
(iv) ANTI-SENSE: No
(v) FRAGMENT TYPE: n/a
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human
(B) STRAIN:
(C) INDIVIDUAL ISOLATE: H4-1BB #1
(D) DEVELOPMENTAL STAGE: Differentiated T-cell
(E) HAPLOTYPE:
(F) TISSUE TYPE:
(G) CELL TYPE: Lymphocytes
(H) CELL LINE:
(I) ORGANELLE:
(vii) IMMEDIATE SOURCE:
(A) LIBRARY: cDNA library
(B) CLONE:
(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT:
(B) MAP POSITION:
(C) UNITS:
(ix) FEATURE:
(A) NAME/KEY: H4-1BB

(B) LOCATION:
(C) IDENTIFICATION METHOD: Similarity to mouse 4-1BB and other members of NGFR superfamily
(D) OTHER INFORMATION:
(x) PUBLICATION INFORMATION:
(A) AUTHORS: Kwon, B.S., and Weissman, S.M.
(B) TITLE: cDNA sequences of two inducible T-cell genes
(C) JOURNAL: Proc. Natl. Acad. Sci. USA
(D) VOLUME: 86
(E) ISSUE:
(F) PAGES: 1963-1967
(G) RELEVANT RESIDUES: all

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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AATCAGCTTT GCTAGTATCA TACCTGTGCC AGATTTCATC                              40

ATG GGA AAC AGC TGT TAC AAC ATA GTA GCC ACT CTG TTG CTG GTC               85
Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr Leu Leu Leu Val
1               5                  10                  15

CTC AAC TTT GAG AGG ACA AGA TCA TTG CAG GAT CCT TGT AGT AAC              130
Leu Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn
            20                  25                  30

TGC CCA GCT GGT ACA TTC TGT GAT AAT AAC AGG AAT CAG ATT TGC              175
Cys Pro Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys
            35                  40                  45

AGT CCC TGT CCT CCA AAT AGT TTC TCC AGC GCA GGT GGA CAA AGG              220
Ser Pro Cys Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg
            50                  55                  60

ACC TGT GAC ATA TGC AGG CAG TGT AAA GGT GTT TTC AGG ACC AGG              265
Thr Cys Asp Ile Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg
            65                  70                  75

AAG GAG TGT TCC TCC ACC AGC AAT GCA GAG TGT GAC TGC ACT CCA              310
Lys Glu Cys Ser Ser Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro
            80                  85                  90
GGG TTT CAC TGC CTG GGG GCA GGA TGC AGC ATG TGT GAA CAG GAT              355
Gly Phe His Cys Leu Gly Ala Gly Cys Ser Met Cys Glu Gln Asp
            95                  100                 105

TGT AAA CAA GGT CAA GAA CTG ACA AAA AAA GGT TGT AAA GAC TGT              400
Cys Lys Gln Gly Gln Glu Leu Thr Lys Lys Gly Cys Lys Asp Cys
            110                 115                 120

TGC TTT GGG ACA TTT AAC GAT CAG AAA CGT GGC ATC TGT CGA CCC              445
Cys Phe Gly Thr Phe Asn Asp Gln Lys Arg Gly Ile Cys Arg Pro
            125                 130                 135

TGG ACA AAC TGT TCT TTG GAT GGA AAG TCT GTG CTT GTG AAT GGG              490
Trp Thr Asn Cys Ser Leu Asp Gly Lys Ser Val Leu Val Asn Gly
            140                 145                 150

ACG AAG GAG AGG GAC GTG GTC TGT GGA CCA TCT CCA GCT GAC CTC              535
Thr Lys Glu Arg Asp Val Val Cys Gly Pro Ser Pro Ala Asp Leu
            155                 160                 165

TCT CCG GGA GCA TCC TCT GTG ACC CCG CCT GCC CCT GCG AGA GAG              580
Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala Pro Ala Arg Glu
            170                 175                 180

CCA GGA CAC TCT CCG CAG ATC ATC TCC TTC TTT CTT GCG CTG ACG              625
Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu Ala Leu Thr
            185                 190                 195
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TCG ACT GCG TTG CTC TTC CTG CTG TTC TTC CTC ACG CTC CGT TTC     670
Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu Arg Phe
                200                 205                 210

TCT GTT GTT AAA CGG GGC AGA AAG AAA CTC CTG TAT ATA TTC AAA     715
Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys
                215                 220                 225

CAA CCA TTT ATG AGA CCA GTA CAA ACT ACT CAA GAG GAA GAT GGC     760
Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
                230                 235                 240

TGT AGC TGC CGA TTT CCA GAA GAA GAA GAA GGA GGA TGT GAA CTG     805
Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
                245                 250                 255

TGAAATGGAA GTCAATAGGG CTGTTGGGAC TTT                           838
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□

CLAIMS:

1. A cDNA encoding for human receptor protein H4-1BB having a nucleotide sequence as shown in SEQ ID NO:1.

2. The cDNA of claim 1, identified as pH4-1BB deposited at the Agricultural Research Service Culture Collection with the accession number NRRL B21131.

3. An isolated DNA, wherein said DNA comprises a nucleotide sequence selected from the group consisting of:

a) nucleotides of SEQ ID NO:3;

b) nucleotides of SEQ ID NO:4;

c) nucleotides of SEQ ID NO:5; and

d) nucleotides of SEQ ID NO:6;

e) an isolated DNA encoding a soluble human 4-1BB polypeptide comprising the extracellular domain of human 4-1BB (amino acids 1-163 of SEQ ID NO:1) or a fragment thereof capable of binding a human 4-1BB-L;

f) an isolated DNA of subparagraph e) wherein said DNA additionally encodes a polypeptide fused to the C-terminus of said soluble human 4-1BB polypeptide;

g) degeneracies of the above that encode for the same amino acid sequence as any of the nucleotide sequences above.

4. An isolated DNA encoding a human 4-1BB polypeptide, wherein said DNA comprises a nucleotide sequence selected from the group consisting of:

a) nucleotides 41-805 of SEQ ID NO:1;

b) nucleotides 110-805 of SEQ ID NO:1; and

c) a nucleotide sequence that is degenerate as a result of the genetic code to a nucleotide sequence of (a) or (b).

5. The receptor protein H4-1BB produced by

a) inserting cDNA including at least nucleotides 110-805 of SEQ ID NO:1 into an appropriate expression vector,

b) transfecting said expression vector into an appropriate transfection host,

c) growing said transfected hosts in appropriate culture media and

d) purifying the receptor protein from said culture media.

6. A protein having the amino acid sequence shown in SEQ ID NO:2.

7. The protein of claim 6 and fragments and derivatives thereof, wherein said fragments and derivatives:

a) can be used as a probe to identify ligands to receptor protein H4-1BB;

b) can be used to stimulate proliferation B-cell's expressing H4-1BB ligands; or

c) can be used to block H4-1BB ligand binding.

8. A fusion protein for detecting cell membrane ligands to human receptor protein H4-1BB, comprising:

a) at least a portion of said receptor protein H4-1BB corresponding to the extracellular portion of said receptor protein H4-1BB such that said portion of said receptor protein H4-1BB binds to said cell membrane ligands; and

b) a detection protein bound to said portion of said receptor protein H4-1BB such that ligand binding can be detected by relative activity assays for said detection protein.

9. The fusion protein of claim 8 wherein said detection protein is alkaline phosphatase.

10. A method of detecting cell membrane ligands to human receptor protein H4-1BB, comprising:

a) providing a fusion protein including:

1) at least a portion of said receptor protein H4-1BB corresponding to the extracellular portion of said receptor protein H4-1BB such that said portion of said receptor protein H4-1BB binds to said cell membrane ligands, and

2) a detection protein bound to said portion of said receptor protein H4-1BB such that ligand binding can be detected by relative activity assays for said detection protein;

b) placing said fusion protein in the presence of a cell suspected to express said receptor protein H4-1BB;

c) washing said cell of any fusion protein not bound to said cell membrane ligands;

d) placing said washed cells in the presence of a substrate for said detection protein and measuring the relative activity of said detection protein.

11. The method of claim 10 wherein said detection protein is alkaline phosphatase.

12. A method of inducing B-cell proliferation *in vitro* comprising the step of treating B-cells that have expressed a ligand to human receptor protein H4-1BB with cells that have expressed receptor protein H4-1BB.

13. A recombinant expression vector comprising a DNA sequence according to claim 1 operably linked to regulatory sequences suitable for expression of said DNA sequence in a host cell.

14. A recombinant expression vector comprising a DNA sequence according to claim 3 operably linked to regulatory sequences suitable for expression of said DNA sequence in a host cell.

15. A recombinant expression vector comprising a DNA sequence according to claim 4 operably linked to regulatory sequences suitable for expression of said DNA sequence in a host cell.

16. A purified human 4-1BB polypeptide comprising the N-terminal amino acid sequence Leu-Gln-Asp-Pro-Cys-Ser-Asn-Cys-Pro-Ala-Gly-Thr-.

17. A purified 4-1BB according to claim 16, comprising an amino acid sequence selected from the group consisting of amino acids 24-255 of SEQ ID NO:2 and amino acids 24-186 of SEQ ID NO:2.

18. A purified 4-1BB according to claim 16, comprising an amino acid sequence that is identical to a sequence selected from the group consisting of amino acids 24-255 of SEQ ID NO:2 and amino acids 24-186 of SEQ ID NO:2, except for conservative amino acid substitution(s).

19. A purified soluble human 4-1BB polypeptide, wherein said polypeptide comprises the extracellular domain of human 4-1BB (amino acids 24-186 of SEQ ID NO:2) or a fragment thereof capable of binding a human 4-1BB ligand.

20. A purified soluble human 4-1BB polypeptide of claim 19, additionally comprising a polypeptide fused to the C-terminus of said human 4-1BB polypeptide.

21. A pharmaceutical composition comprising an effective amount of soluble human 4-1BB of claim 16 in admixture with a suitable diluent, carrier, or excipient.

22. An antibody that is specifically immunoreactive with a human 4-1BB polypeptide of claim 16.

23. The use of cells that have expressed receptor protein H4-1BB to treat B-cells that have expressed a ligand to human receptor protein H4-1BB to induce B-cell proliferation.

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Fig. 1

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ATGTCCATGA ACTGCTGAGT GGATAAACAG CACGGGATAT CTCTGTCTAA        -96
AGGAATATTA CTACACCAGG AAAAGGACAC ATTCGACAAC AGGAAAGGAG        -46
CCTGTCACAG AAAACCACAG TGTCCTGTGC ATGTGACATT TCGCC             -1

ATG GGA AAC AAC TGT TAC AAC GTG GTG GTC ATT GTG CTG CTG CTA    45
Met Gly Asn Asn Cys Tyr Asn Val Val Val Ile Val Leu Leu Leu

GTG GGC TGT GAG AAG GTG GGA GCC GTG CAG AAC TCC TGT GAT AAC    90
Val Gly Cys Glu Lys Val Gly Ala Val Gln Asn Ser Cys Asp Asn

TGT CAG CCT GGT ACT TTC TGC AGA AAA TAC AAT CCA GTC TGC AAG   135
Cys Gln Pro Gly Thr Phe Cys Arg Lys Tyr Asn Pro Val Cys Lys
                          H4-1BB FI

AGC TGC CCT CCA AGT ACC TTC TCC AGC ATA GGT GGA CAG CCG AAC   180
Ser Cys Pro Pro Ser Thr Phe Ser Ser Ile Gly Gly Gln Pro Asn
                          H4-1BB FII

TGT AAC ATC TGC AGA GTG TGT GCA GGC TAT TTC AGG TTC AAG AAG   225
Cys Asn Ile Cys Arg Val Cys Ala Gly Tyr Phe Arg Phe Lys Lys

TTT TGC TCC TCT ACC CAC AAC GCG GAG TGT GAG TGC ATT GAA GGA   270
Phe Cys Ser Ser Thr His Asn Ala Glu Cys Glu Cys Ile Glu Gly

TTC CAT TGC TTG GGG CCA CAG TGC ACC AGA TGT GAA AAG GAC TGC   315
Phe His Cys Leu Gly Pro Gln Cys Thr Arg Cys Glu Lys Asp Cys

AGG CCT GGC CAG GAG CTA ACG AAG CAG GGT TGC AAA ACC TGT AGC   360
Arg Pro Gly Gln Glu Leu Thr Lys Gln Gly Cys Lys Thr Cys Ser
                          H4-1BB RI

TTG GGA ACA TTT AAT GAC CAG AAC GGT ACT GGC GTC TGT CGA CCC   405
Leu Gly Thr Phe Asn Asp Gln Asn Gly Thr Gly Val Cys Arg Pro
                          H4-1BB RII

TGG ACG AAC TGC TCT CTA GAC GGA AGG TCT GTG CTT AAG ACC GGG   450
Trp Thr Asn Cys Ser Leu Asp Gly Arg Ser Val Leu Lys Thr Gly

ACC ACG GAG AAG GAC GTG GTG TGT GGA CCC CCT GTG GTG AGC TTC   495
Thr Thr Glu Lys Asp Val Val Cys Gly Pro Pro Val Val Ser Phe

TCT CCC AGT ACC ACC ATT TCT GTG ACT CCA GAG GGA GGA CCA GGA   540
Ser Pro Ser Thr Thr Ile Ser Val Thr Pro Glu Gly Gly Pro Gly

GGG CAC TCC TTG CAG GTC CTT ACC TTG TTC CTG GCG CTG ACA TCG   585
Gly His Ser Leu Gln Val Leu Thr Leu Phe Leu Ala Leu Thr Ser

GCT TTG CTG CTG GCC CTG ATC TTC ATT ACT CTC CTG TTC TCT GTG   630
Ala Leu Leu Leu Ala Leu Ile Phe Ile Thr Leu Leu Phe Ser Val

CTC AAA TGG ATC AGG AAA AAA TTC CCC CAC ATA TTC AAG CAA CCA   675
Leu Lys Trp Ile Arg Lys Lys Phe Pro His Ile Phe Lys Gln Pro

TTT AAG AAG ACC ACT GGA GCA GCT CAA GAG GAA GAT GCT TGT AGC   720
Phe Lys Lys Thr Thr Gly Ala Ala Gln Glu Glu Asp Ala Cys Ser
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D

Fig.1 (cont'd)

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TGC CGA TGT CCA CAG GAA GAA GAA GGA GGA GGA GGA GGC TAT GAG   765
Cys Arg Cys Pro Gln Glu Glu Glu Gly Gly Gly Gly Gly Tyr Glu
 ●       ●

CTG TGA                                                      771
Leu ---
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```
TGTACTATCC TAGGAGATGT GTGGGCCGAA ACCGAGAAGC ACTAGGACCC   821
CACCATCCTG TGGAACAGCA CAAGCAACCC CACCACCCTG TTCTTACACA   871
TCATCCTAGA TGATGTGTGG GCGCGCACCT CATCCAAGTC TCTTCTAACG   921
CTAACATATT TGTCTTTACC TTTTTTAAAT CTTTTTTTAA ATTTAAATTT   971
TATGTGTGTG AGTGTTTTGC CTGCCTGTAT GCACACGTGT GTGTGTGTGT  1021
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CCATAAGAAC TGGAGTTATG GATGGCTGTG AGCCGGNNNG ATAGGTCGGG  1121
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GATATTTCGG GAATTGTAGA GATTGTCCTG ACACCCTTCT AGTTAATGAT  1221
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TTACAAAAGT AATTAGTTCT TGCTTTCAGC CTCCAAGCTT CTGCTAGTCT  2021
ATGGCAGCAT CAAGGCTGGT ATTTGCTACG GCTGACCGCT ACGCCGCCGC  2071
AATAAGGGTA CTGGGCGGCC CGTCGAAGGC CCTTTGGTTT CAGAAACCCA  2121
AGGCCCCCCT CATACCAACG TTTCGACTTT GATTCTTGCC GGTACGTGGT  2171
GGTGGGTGCC TTAGCTCTTT CTCGATAGTT AGAC                   2205
```

D

Fig. 2a

human homologue of mouse 4-1bb
h4-1bb Length 838

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   1  AATCAGCTTT GCTAGTATCA TACCTGTGCC AGATTTCATC ATGGGAAACA
  51  GCTGTTACAA CATAGTAGCC ACTCTGTTGC TGGTCCTCAA CTTTGAGAGG
 101  ACAAGATCAT TGCAGGATCC TTGTAGTAAC TGCCCAGCTG GTACATTCTG
 151  TGATAATAAC AGGAATCAGA TTTGCAGTCC CTGTCCTCCA AATAGTTTCT
 201  CCAGCGCAGG TGGACAAAGG ACCTGTGACA TATGCAGGCA GTGTAAAGGT
 251  GTTTTCAGGA CCAGGAAGGA GTGTTCCTCC ACCAGCAATG CAGAGTGTGA
 301  CTGCACTCCA GGGTTTCACT GCCTGGGGGC AGGATGCAGC ATGTGTGAAC
 351  AGGATTGTAA ACAAGGTCAA GAACTGACAA AAAAAGGTTG TAAAGACTGT
 401  TGCTTTGGGA CATTTAACGA TCAGAAACGT GGCATCTGTC GACCCTGGAC
 451  AAACTGTTCT TTGGATGGAA AGTCTGTGCT TGTGAATGGG ACGAAGGAGA
 501  GGGACGTGGT CTGTGGACCA TCTCCAGCTG ACCTCTCTCC GGGAGCATCC
 551  TCTGTGACCC CGCCTGCCCC TGCGAGAGAG CCAGGACACT CTCCGCAGAT
 601  CATCTCCTTC TTTCTTGCGC TGACGTCGAC TGCGTTGCTC TTCCTGCTGT
 651  TCTTCCTCAC GCTCCGTTTC TCTGTTGTTA AACGGGGCAG AAAGAAACTC
 701  CTGTATATAT TCAAACAACC ATTTATGAGA CCAGTACAAA CTACTCAAGA
 751  GGAAGATGGC TGTAGCTGCC GATTTCCAGA AGAAGAAGAA GGAGGATGTG
 801  AACTGTGAAA TGGAAGTCAA TAGGGCTGTT GGGACTTT
```

Fig. 2b

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   1  MGNSCYNIVA TLLLVLNFER TRSLQDPCSN CPAGTFCDNN RNQICSPCPP
  51  NSFSSAGGQR TCDICRQCKG VFRTRKECSS TSNAECDCTP GFHCLGAGCS
 101  MCEQDCKQGQ ELTKKGCKDC CFGTFNDQKR GICRPWTNCS LDGKSVLVNG
 151  TKERDVVCGP SPADLSPGAS SVTPPAPARE PGHSPQIISF FLALTSTALL
 201  FLLFFLTLRF SVVKRGRKKL LYIFKQPFMR PVQTTQEEDG CSCRFPEEEE
 251  GGCEL
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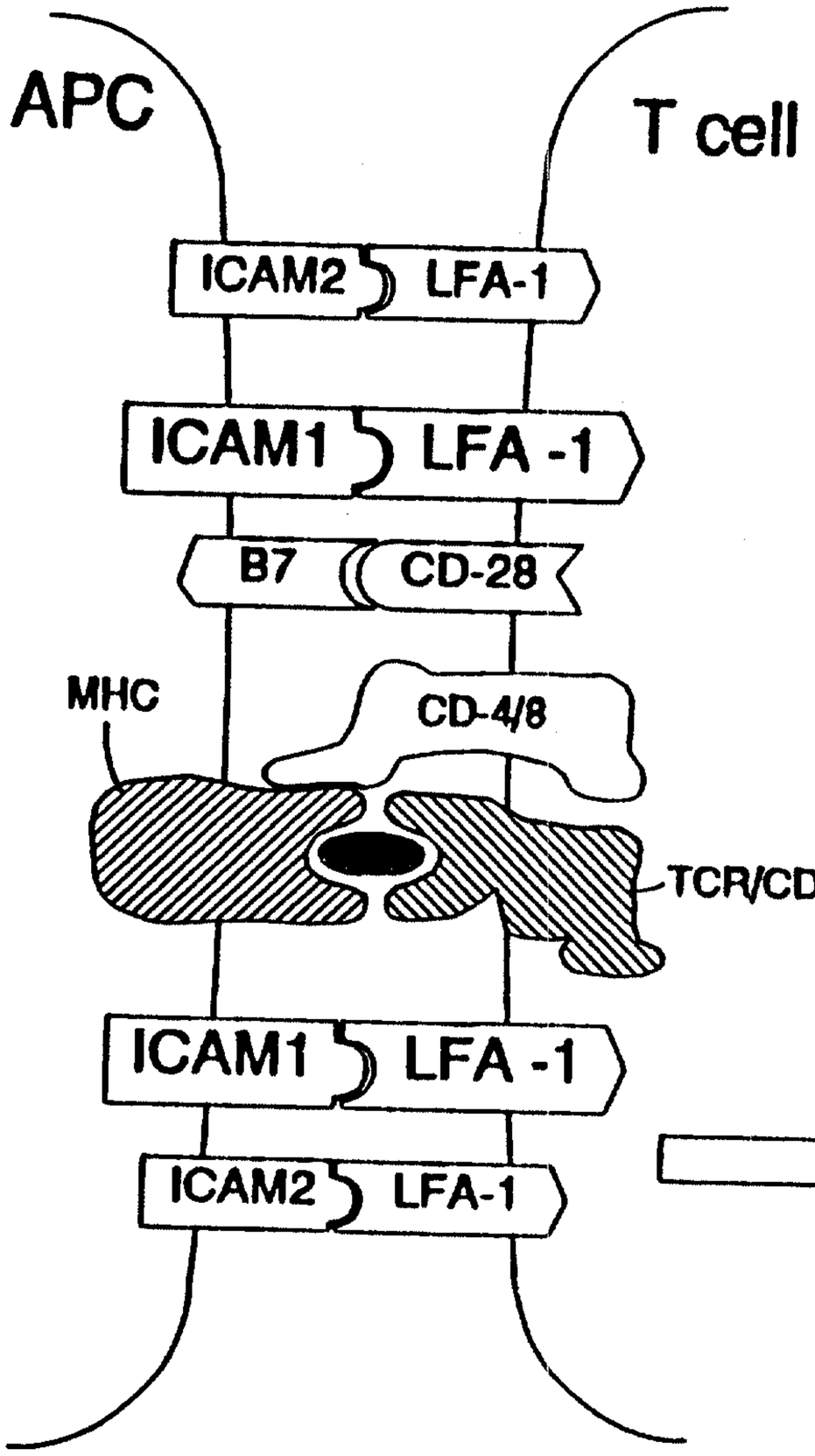
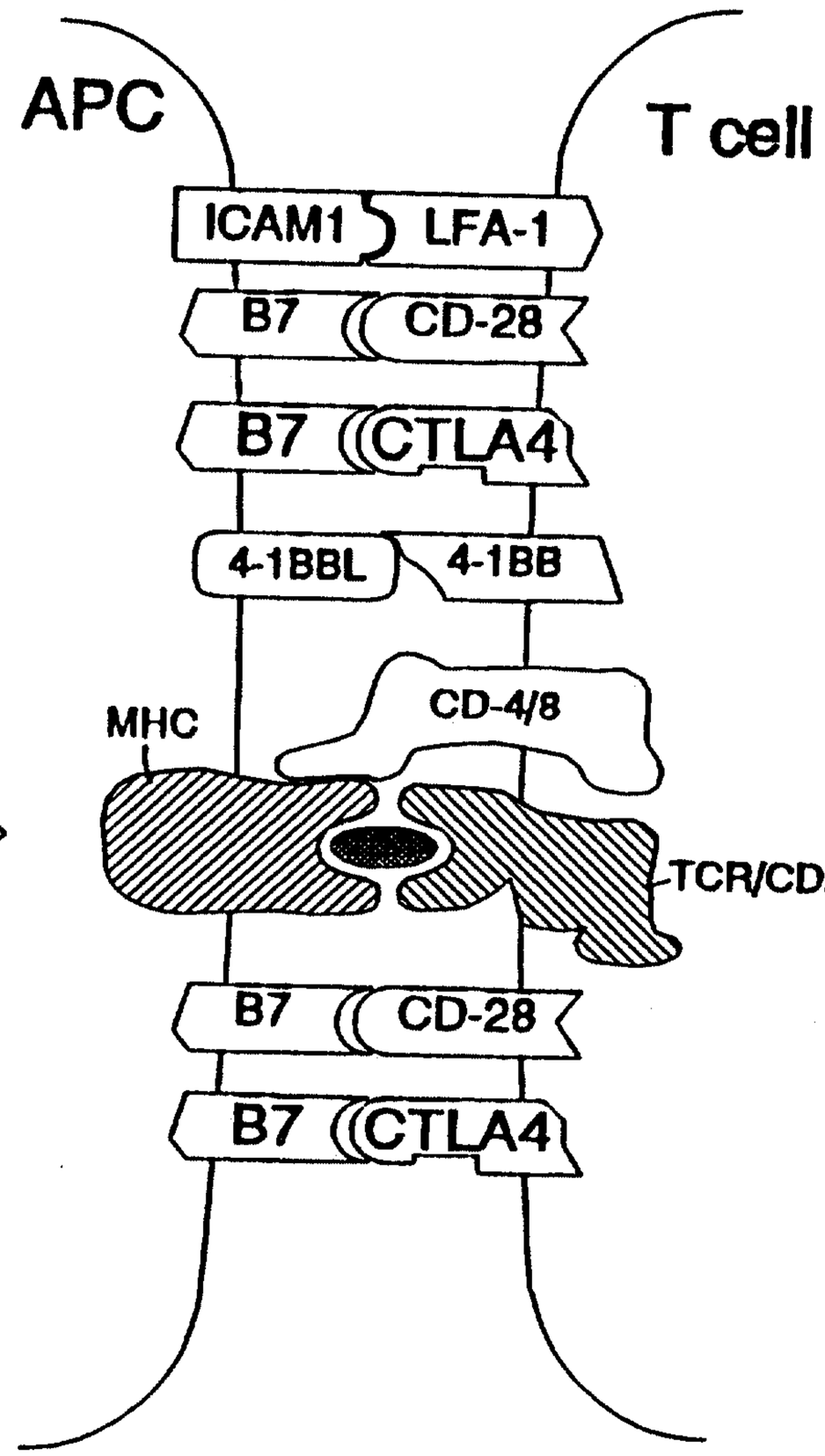
D



COGNITIVE PHASE
early activation

Fig. 3a



PROLIFERATION
CLONAL EXPANSION
late activation

Fig. 3b

D

2172165

NORMAL T-CELL ACTIVATION PATHWAY

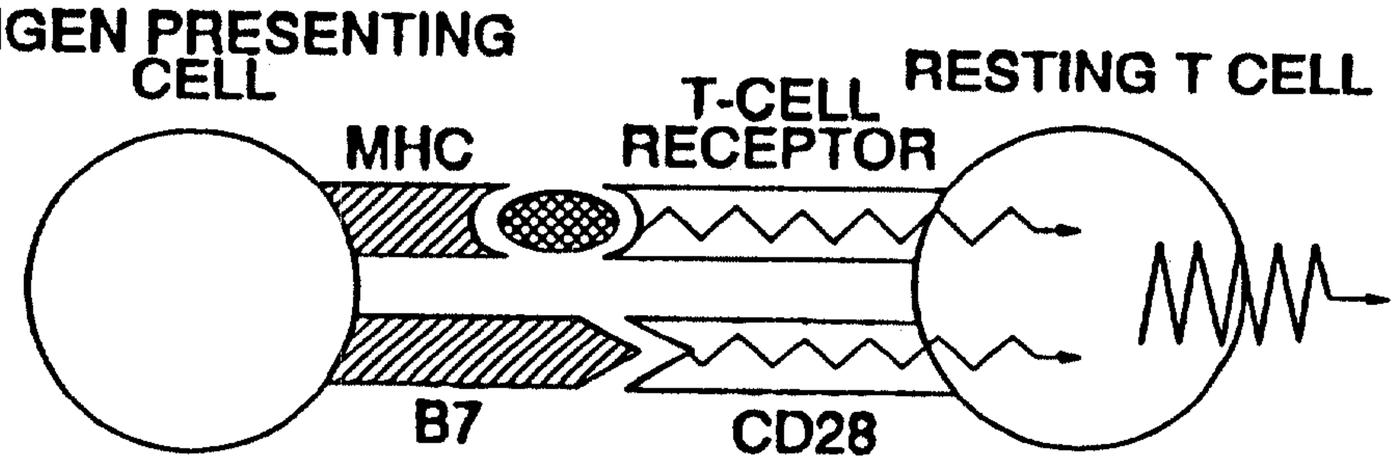


Fig. 4a

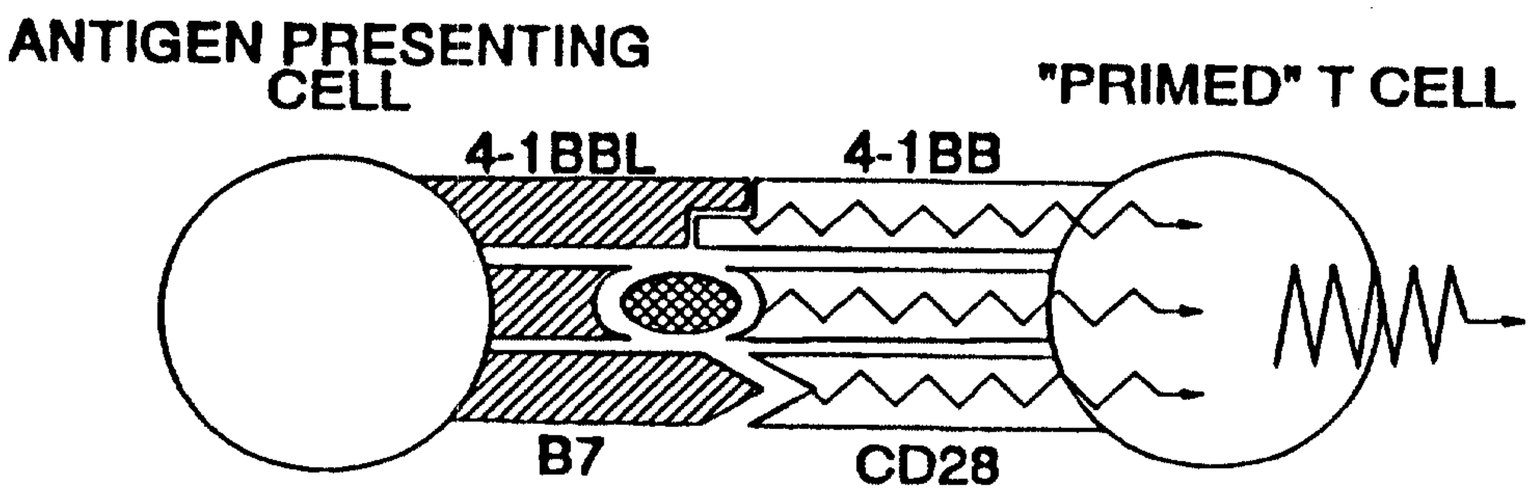


Fig. 4b

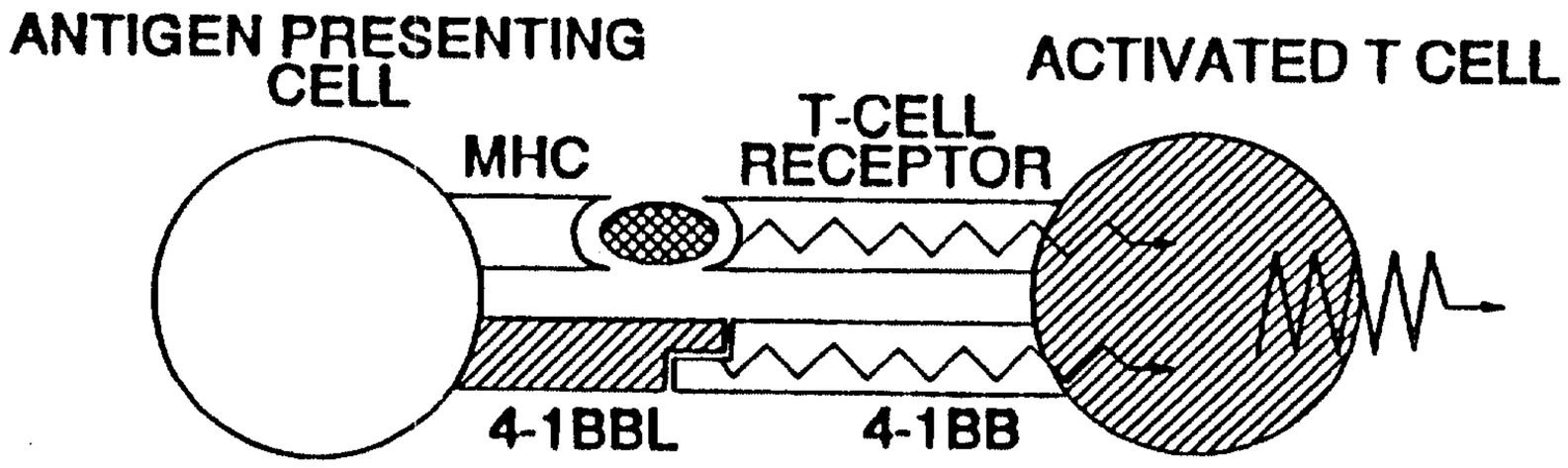


Fig. 4c

D

2172165

BLOCKING STEPS IN T-CELL ACTIVATION PATHWAY

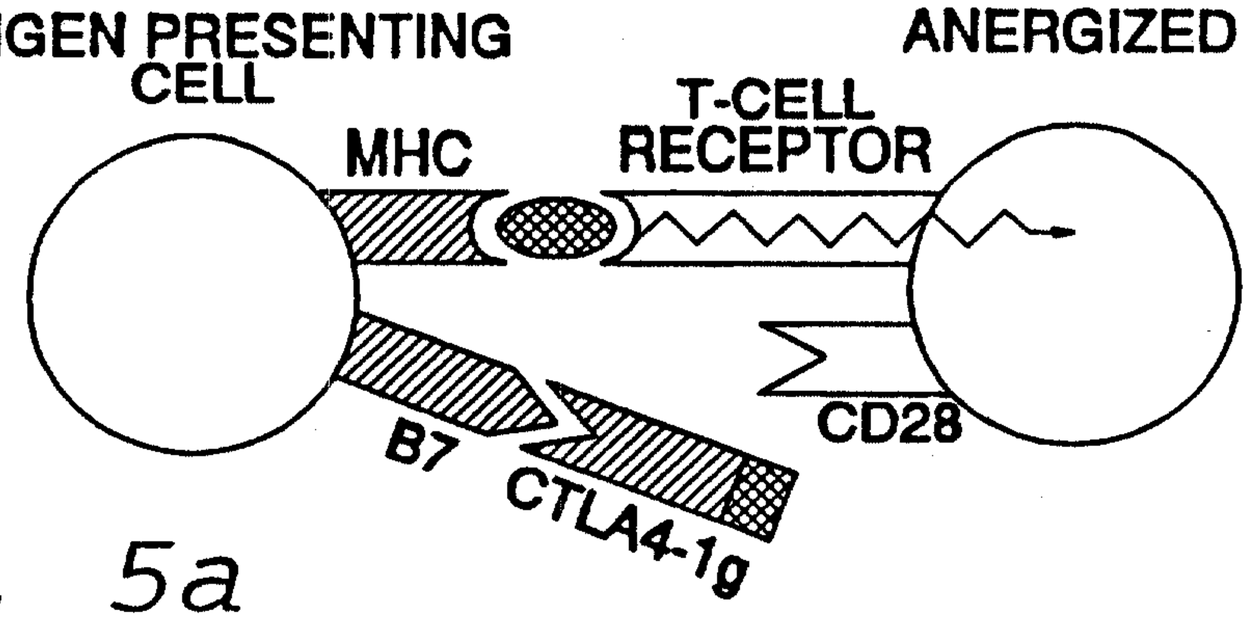


Fig. 5a

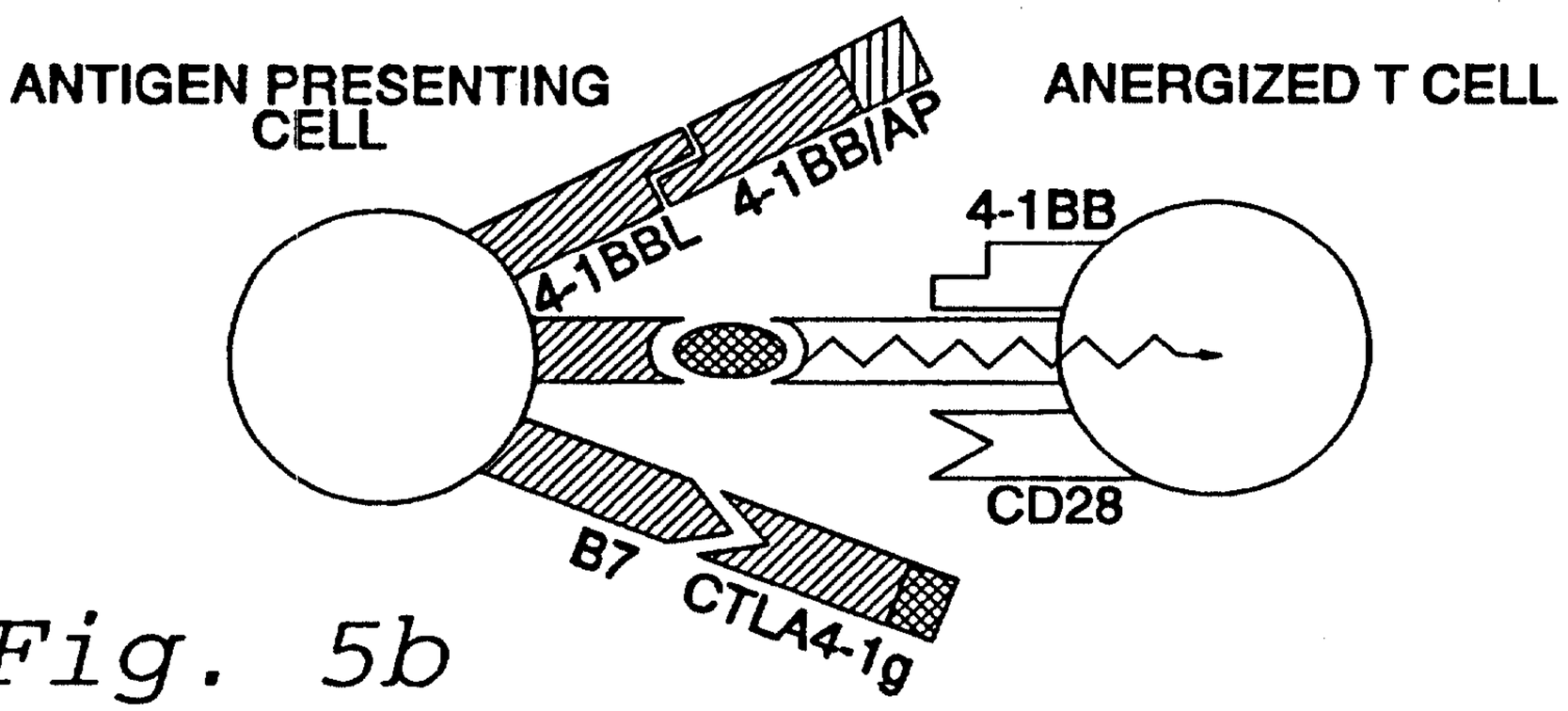


Fig. 5b

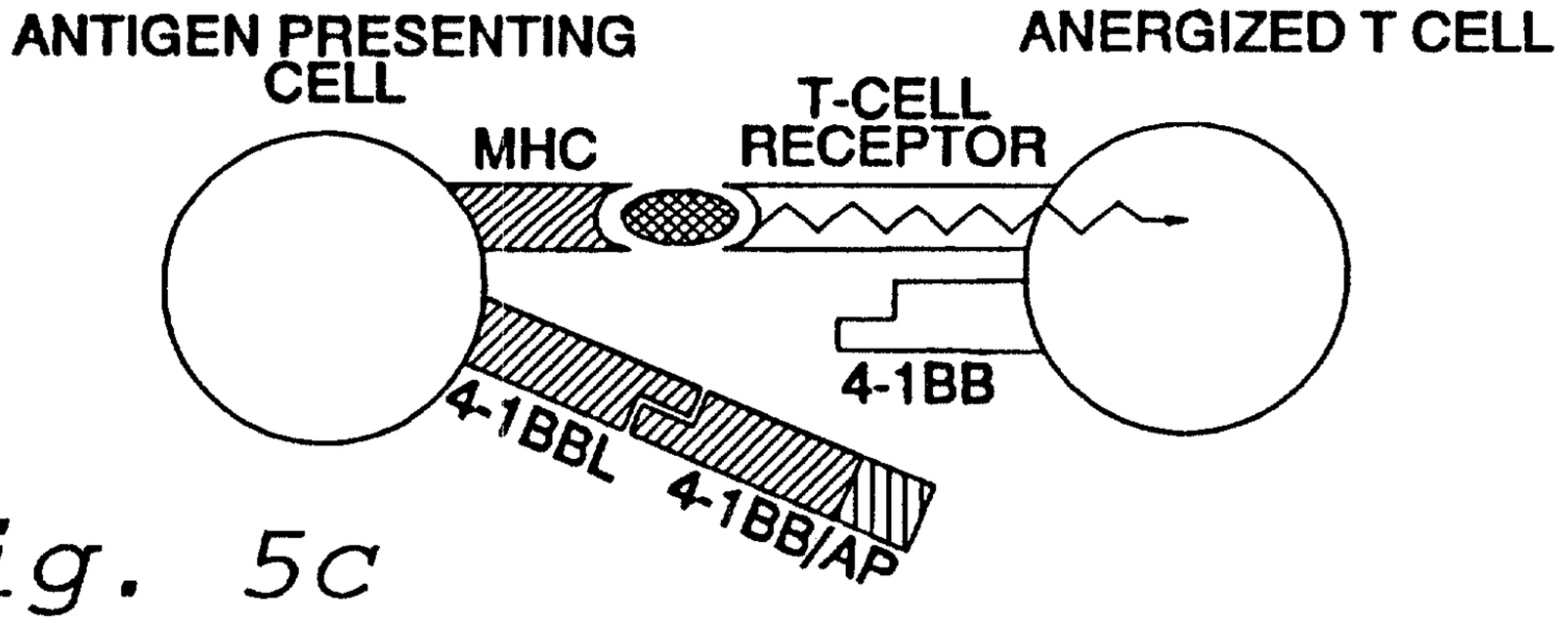


Fig. 5c

D