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(12) (19) (CA) **Brevet-Patent**

(11) (21) (C) **2,135,277**

(86) 1993/05/07
(87) 1993/11/25
(45) 2001/04/24

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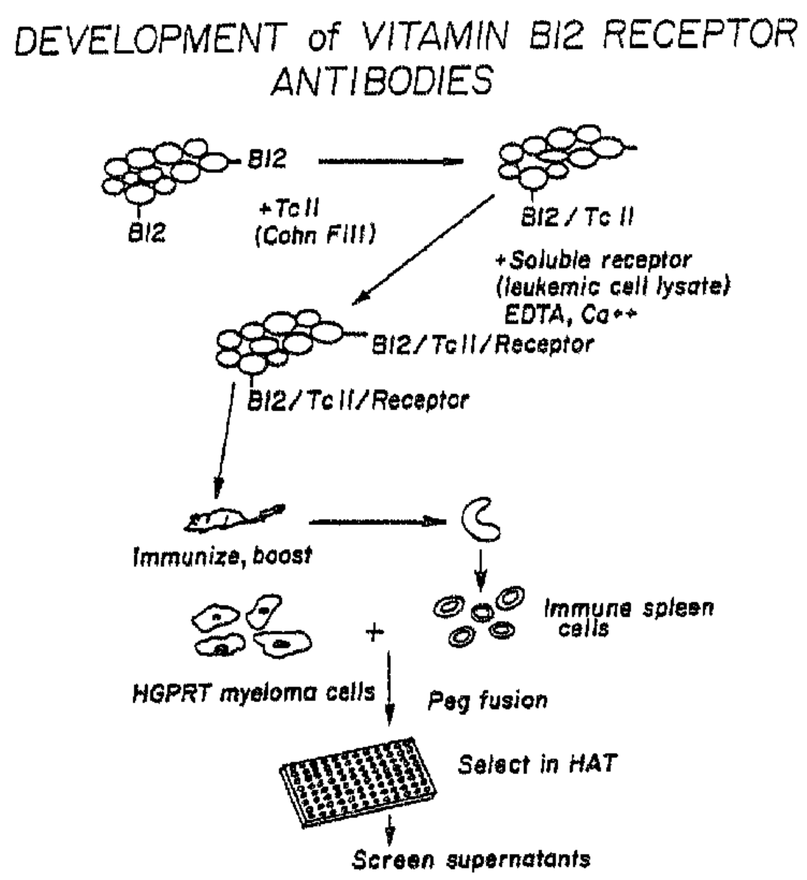
(51) Int.Cl.[5] C12P 21/08, A61K 39/395, C07K 16/28
(30) 1992/05/08 (07/880,540) US

(54) **AGENTS CONTRE LE RECEPTEUR DE LA VITAMINE B12 ET DE LA TRANSCOBALAMINE II**

(54) **ANTI-RECEPTOR AGENTS TO THE VITAMIN B12/TRANSCOBALAMIN II RECEPTOR**



(57) L'invention concerne des agents antirécepteurs dirigés contre le récepteur de la vitamine B12/transcobalamine II. Lesdits agents antirécepteurs s'opposent à ou modulent le récepteur de la vitamine B12/transcobalamine II en provoquant la déplétion cellulaire de la vitamine B12, empêchant ou stoppant ainsi la division cellulaire. Les agents antirécepteurs selon l'invention comprennent des protéines (anticorps ou dérivés d'anticorps), des peptides et des petites molécules organiques. Dans un mode préféré de réalisation, l'agent antirécepteur est un anticorps dirigé contre le récepteur de la vitamine B12/transcobalamine II.

(57) There is disclosed anti-receptor agents to the vitamin B12/transcobalamin II receptor. The anti-receptor agents antagonize or modulate the vitamin B12/transcobalamin II receptor, causing cellular depletion of vitamin B12, thus preventing or inhibiting cell division. Anti-receptor agents of the present invention include proteins (such as antibodies and antibody derivatives), peptides and small organic molecules. In a preferred embodiment, the anti-receptor agent is an antibody to the vitamin B12/transcobalamin II receptor.

2135277

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12P 21/08, A61K 39/395
// C12N 5/20

A1

(11) International Publication Number: WO 93/23557

(43) International Publication Date: 25 November 1993 (25.11.93)

(21) International Application Number: PCT/US93/04341

(22) International Filing Date: 7 May 1993 (07.05.93)

(30) Priority data:
07/880,540 8 May 1992 (08.05.92) US

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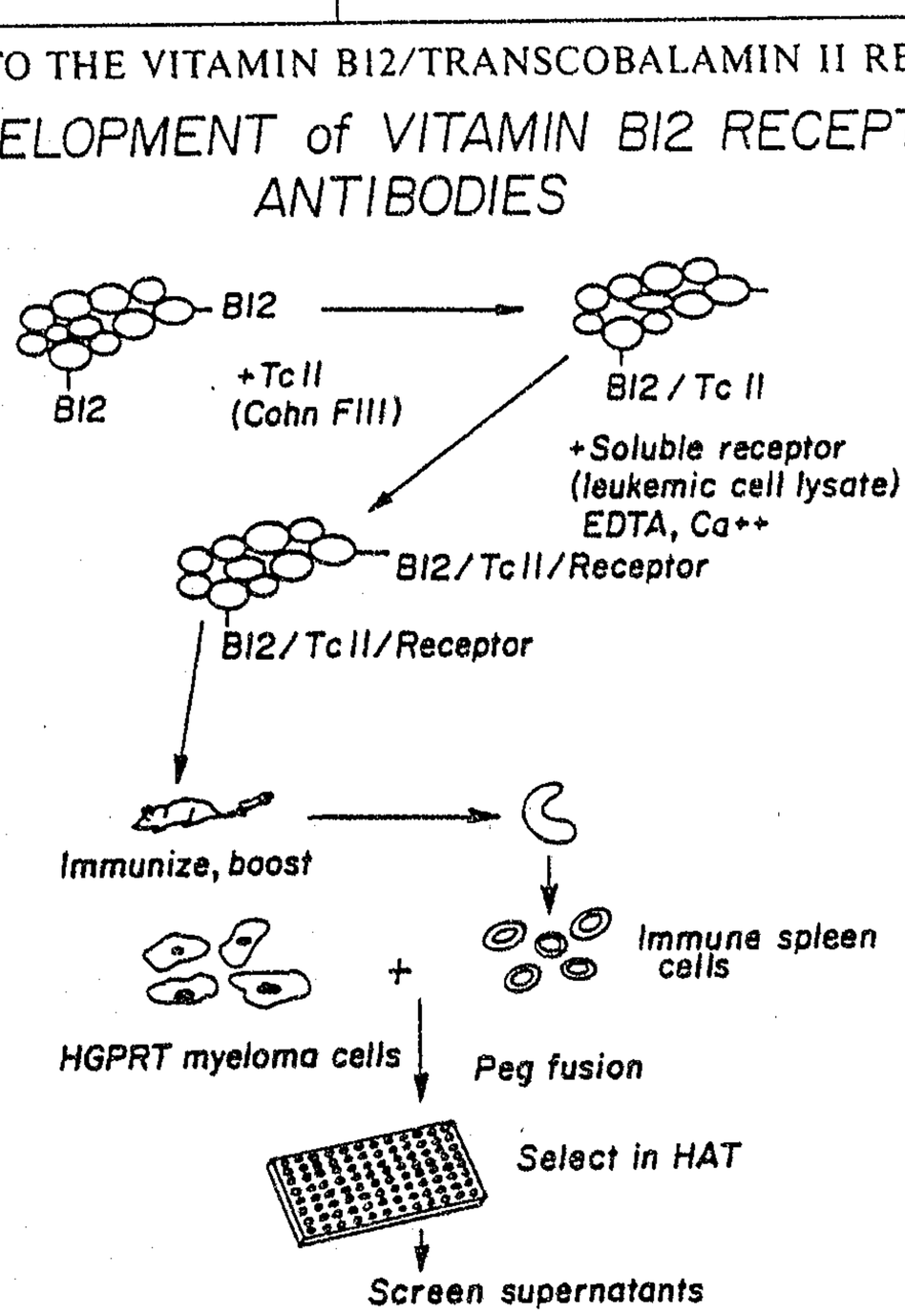
(81) Designated States: AU, CA, JP, KR, NO, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ANTI-RECEPTOR AGENTS TO THE VITAMIN B12/TRANSCOBALAMIN II RECEPTOR



(57) Abstract

There is disclosed anti-receptor agents to the vitamin B12/transcobalamin II receptor. The anti-receptor agents antagonize or modulate the vitamin B12/transcobalamin II receptor, causing cellular depletion of vitamin B12, thus preventing or inhibiting cell division. Anti-receptor agents of the present invention include proteins (such as antibodies and antibody derivatives), peptides and small organic molecules. In a preferred embodiment, the anti-receptor agent is an antibody to the vitamin B12/transcobalamin II receptor.

Description

ANTI-RECEPTOR AGENTS TO THE VITAMIN B12/TRANSCOBALAMIN II RECEPTOR

Technical Field

The present invention generally relates to anti-receptor agents to the vitamin B12/transcobalamin II receptor, and more specifically, to agents which can cause the cellular depletion of vitamin B12 by antagonizing or modulating the vitamin B12/transcobalamin II receptor, thereby inhibiting cell division of normal or neoplastic cells.

Background of the Invention

Experimental *in vitro* data, pre-clinical animal models, and patient studies have demonstrated the requirement of proliferating normal and neoplastic cells for vitamin B12. This nutrient is a co-factor for enzymes necessary in cell division, as well as cellular metabolism. The nutrient is derived from dietary intake and is transported throughout the body complexed to transport proteins. The complex of transport protein and vitamin B12 is recognized by a cellular receptor which internalizes the complex and releases the vitamin intracellularly. The overall process has been recently reviewed (*GUT 31*:59, 1991). Vitamin B12 is taken in through the diet. Binding proteins in the saliva (R-binder) and gut (intrinsic factor-(IF)) complex B12 after release from endogenous binding proteins by action of enzymes and low pH in the stomach. B12 is transferred across the intestinal epithelium in a receptor specific fashion to transcobalamin II (TcII). The vitamin B12/transcobalamin II complex is then transported throughout the body and recognized by receptors present on dividing cells, internalized and released within the cell where it is utilized by certain enzymes as a co-factor.

The high affinity receptor in dividing tissues or cells responsible for internalization of B12 recognizes transcobalamin II complexed with vitamin B12. The B12/TcII receptor recognizes only the vitamin B12/transcobalamin complex and not the serum transport protein or the vitamin alone. The receptor is undetectable on non-dividing cells; the mechanism for supplying non-dividing cells with vitamin B12 is poorly understood. However, it is known that more vitamin B12 is required during cell division than during metabolism, and that the B12/TcII receptor is the only high affinity means for cellular uptake of vitamin B12 during cell division. When stimulated to divide, cells demonstrate transient expression of

this receptor leading to B12 uptake which precedes actual DNA synthesis (*J. Lab. Clin. Med. 103*:70, 1984). Vitamin B12 receptor levels may be measured by binding of ^{57}Co-cobalamin complexed to transcobalamin II (present in serum) on replicate cultures grown in chemically defined medium without serum. No receptor mediated uptake occurs in the absence of carrier protein.

Dividing cells, induced to differentiate, loose receptor expression and no longer take up vitamin B12. More importantly, leukemic cells, deprived of vitamin B12, will stop dividing and die (*Acta Haemat. 81*:61, 1989). In a typical experiment, leukemic cell cultures were deprived of serum for 3 days, and then supplemented either with serum (a source of B12) or a non-metabolizable analogue of B12 and cultured up to five days. Cell cultures supplemented with vitamin B12 continued to grow whereas those deprived of the active nutrient stopped growing and died.

Based on these observations, it has been suggested that whole body deprivation of vitamin B12 may be useful in the treatment of cancer. Moreover, because of the critical role played by vitamin B12-containing enzymes in cell division, it is believed that B12 deprivation may be used in combination with chemotherapeutic drugs which inhibit cellular replication. For example, when vitamin B12 depletion was combined with methotrexate, the two modalities together were more efficient in depleting folate levels in leukemic cells than either alone (*FASEB J.* 4:1450, 1990; *Arch. Biochem. Biophys.* 270:729, 1989; *Leukemia Research* 15:165, 1991). Folates are precursors in the production of DNA and proteins. In typical experiments, cultures of leukemic cells were exposed to nitrous oxide for several hours to convert the active form of endogenous B12 to an inactive form. Replicate cultures were then left without further treatment, or additionally treated with methotrexate. Cellular folate levels were measured three days later. Cells treated with the combination (i.e., both methotrexate and inactive B12) showed a more striking decrease in cellular folate levels than with either of the two approaches alone. This combination also results in a higher cell kill *in vitro*. When this approach was applied to the treatment of highly aggressive leukemia/lymphoma in animal models (*Am. J. Haematol. 34*:128,1990; *Anticancer Res.* 6:737, 1986; *Cancer Chemother. Pharmacol. 17*:114, 1986; *Br. J. Cancer 50*:793, 1984), additive or synergy of anti-tumor action was observed, resulting in prolonged remissions and cures. The following Table 1 summarizes the observed additive or synergistic results.

Table 1

Vitamin B12 Depletion (Nitrous Oxide) in Combination Therapy

Study	Drugs Used in Combination with Vitamin B12 Depletion	Therapeutic Results
Myelocytic leukemia/rats	cycloleucine	additive
	5-FU	additive
	methotrexate	synergistic
Acute leukemia/rats	5-FU	additive
Acute leukemia/rats	methotrexate	synergistic
Acute leukemia/rats	cycloleucine	synergistic

A key finding in the experiments described above was that short-term (hours to days), whole body depletion of vitamin B12 can act synergistically with chemotherapeutic drugs (such as methotrexate and 5-FU) to inhibit tumor growth and cure animals of leukemia/lymphoma. Despite synergistic anti-tumor activity, there was no toxicity attributable to the short-term vitamin B12 depletion for proliferating normal cells. This combination therapy was demonstrated in multiple animal models. Observations in patients have indicated that long-term (months to years) vitamin B12 depletion is required to produce significant normal tissue toxicity. Even in those cases, subsequent infusion of B12 can readily reverse symptomology (*Br. J. Cancer* 5:810, 1989).

Because of the promise of this therapeutic approach, various methods have been sought to efficiently and controllably perform a temporary depletion of vitamin B12. Such methods, however, affect all of the body's stores of vitamin B12. They include dietary restriction, high doses of cobalamin analogues (non-metabolizable-competitive antagonists which act as enzyme inhibitors), and nitrous oxide (transformation of B12 to inactivate form). These different methods have been used in culture systems and in animals to deplete vitamin B12. The most efficient and the most utilized method has been the inhalation of nitrous oxide (laughing gas). Animals are maintained typically under an atmosphere of 50% to 70% of nitrous oxide for periods from a few hours to a few days, causing the conversion of endogenous B12 into an inactive form. This methodology has been utilized in combination with drugs for therapy of leukemia/lymphoma. A further method for vitamin B12 depletion involves

infusion of a non-metabolizable analog of B12 which essentially dilutes out the active form. This form of therapy is not specific for dividing cells but affects liver dependent metabolic processes. Another approach includes restricting the dietary intake of vitamin B12. This method, however, requires very long periods of dietary restriction and is offset by hepatic storage of vitamin B12. All of these methods suffer from problems of specificity, since they affect both B12-dependent growth as well as basal metabolism, and therefore are not particularly suited to the development of anti-proliferative pharmaceutical products.

Accordingly, there is a need in the art for agents which will cause the cellular depletion of vitamin B12, and which selectively affect dividing cells. The present invention fulfills this need, and provides further related advantages.

Summary of the Invention

The present invention discloses anti-receptor agents which antagonize or modulate the vitamin B12/transcobalamin II receptor. Such agents cause cellular depletion of vitamin B12 by interfering with receptor recognition of the vitamin B12/transcobalamin II complex, thus preventing or inhibiting cell division. Anti-receptor agents which antagonize (e.g., block) the vitamin B12/transcobalamin II receptor function by competitively binding to the receptor, thereby preventing cellular uptake of vitamin B12. Alternatively, the antagonists may sterically hinder recognition of the complex by the receptor by binding sufficiently near the receptor, and thus prevent cellular uptake of vitamin B12. Anti-receptor agents of the present invention which modulate the vitamin B12/transcobalamin II receptor, bind to the receptor and cause the removal or clearing of the receptor for a period of time, and thus inhibits cellular uptake of vitamin B12. Anti-receptor agents of the present invention include proteins (e.g., antibodies and antibody derivatives), peptides and small organic molecules that can antagonize or modulate the vitamin B12/transcobalamin II receptor and cause the cellular depletion of vitamin B12, thereby inhibiting cell division of normal or neoplastic cells.

In one embodiment of the present invention, an anti-receptor agent to the vitamin B12/transcobalamin II receptor is disclosed. This agent is capable of competitively antagonizing or modulating the receptor to prevent cellular uptake of vitamin B12. In a preferred embodiment, the anti-receptor agent is an antibody (or derivative thereof) to the vitamin B12/transcobalamin II receptor.

In a further embodiment, the present invention discloses a method for inhibiting cell division in warm-blooded animals by administering to the animal

an anti-receptor agent to the vitamin B12/transcobalamin II receptor, wherein the agent is capable of antagonizing or modulating the receptor to prevent or inhibit cellular uptake of vitamin B12.

In yet a further embodiment, a method is disclosed for preventing or inhibiting cellular uptake of vitamin B12 in warm-blooded animals by administering to the animal an anti-receptor agent to the vitamin B12/transcobalamin II receptor, wherein the agent is capable of antagonizing or modulating the receptor.

Description of the Drawings

Figure 1 illustrates the development of vitamin B12 transcobalamin II receptor antibodies through the production of murine monoclonal antibodies to the receptor using immunogens consisting of a solid phase, affinity sorbent for the receptor to elicit antibodies, followed by hybridoma generation and screening using both binding and functional assays.

Figure 2 illustrates the administration of vitamin B12 anti-receptor antibodies (i.e., infusions every 2 to 3 days) to maintain serum concentrations above the requisite level (indicated by tri-partite line) for complete or near complete receptor blockade. The requisite concentration is determined by the amount of antibody required to block ≥90% of vitamin B12 uptake into cultured leukemic cells (K562) as assessed by functional assays.

Figure 3 illustrates assessment of vitamin B12/transcobalamin II receptor modulation by vitamin B12 anti-receptor antibodies. 100 ng/ml of antibody is incubated with 1 million Raji Burkitt lymphoma cells. Half of the cells are held at 40°C and half transferred to 37°C after washing. Thirty minutes to 2 hours later, samples are assessed for residual mouse Ig bound to cells by flow cytometry (MFI = mean fluorescence intensity of all cells).

Detailed Description of the Invention

The present invention discloses anti-receptor agents to the vitamin B12/transcobalamin II ("B12/TcII") receptor. Within the context of the present invention, "anti-receptor agents" cause the cellular depletion of vitamin B12 by acting as competitive antagonists or as modulating agents to the B12/TcII receptor. Competitive antagonists are agents which competitively bind to (or sterically hinder) the B12/TcII receptor, thereby preventing or inhibiting cellular uptake of vitamin B12. Modulating agents bind to the B12/TcII receptor, and result in the clearing or removal of the receptor for a period of time (generally hours), followed

by regeneration and re-expression of the receptor (assuming the modulating agent is no longer present). Anti-receptor agents of the present invention include proteins (such as antibodies), peptides and small organic compounds.

The present invention is directed to novel methods for depletion of cellular vitamin B12 in warm-blooded animals by, for example, administering to the animal an anti-receptor antibody to the B12/TcII complex, wherein the antibody competitively antagonizes or modulates the receptor to prevent or inhibit cellular uptake of vitamin B12. Due to the low expression of the B12/TcII receptor (e.g., only a few thousand per cell) and the need to generate functional antibodies that elicit a biological response (e.g., cause cellular depletion of vitamin B12), novel methods of immunization are described herein for eliciting functional antibodies (e.g., combining affinity enrichment of the receptor together with the use of solid phase immunogens to enhance the response to these weakly immunogenic and poorly expressed antigens). Only a portion of these anti-receptor antibodies function as antagonists or to modulate cellular receptors. Appropriate antibodies may be identified by bioassays as illustrated in Figure 1.

Antibodies (and antibody derivatives) of various specificities can be generated to the B12/TcII receptors. Antibodies of the present invention can fall into several functional and specificity categories and have different pharmaceutical applications. Anti-receptor antibodies to the B12/TcII receptor include those which (1) bind but do not produce a biological response; (2) cross-link, modulate and clear the surface of receptors and, if the concentration of modulating antibody is maintained in the patient's circulation at sufficient levels, modulate any newly synthesized receptor when it is re-expressed (IgM antibodies typically are the most efficient modulating agents); and (3) function as competitive antagonists for vitamin B12 binding. Each of these types of antibodies may be distinguished by specific binding or functional assays in a series of screens, beginning with primary screens using initial hybridoma cultures, through secondary screens of clones, and finally to more labor intensive assays of final, stably secreting clones. (*See* Examples 1 and 2 below). Murine antibodies that are generated are used to demonstrate the comparability of antibody mediated depletion to nitrous oxide conversion of vitamin B12, and in causing the death of tumor cells in culture.

The pharmaceutical application of murine antibodies, however, is limited by the potential for anti-murine antiglobulin responses in patients (i.e., immunogenicity). Thus, murine antibodies require genetic manipulation for their conversion to human-mouse chimeras. Numerous methods exist for conversion of murine antibodies to chimeras in which the heavy and light chain constant regions

are substituted with human versions or in which all but the CDRs (complementary determining regions) are substituted with their human equivalents. (*See Biochem. J. 281*:317, 1992; *Proc. Nat. Acad. Sci. USA 86*:10029, 1989; *Methods Enzymol. 178*:515, 1989; *Cancer Res. 51*:181, 1991; *Biotechniques 7*:360, 1989; *J. Immunol. 143*:3589, 1989; *Int. J. Cancer 44*:424, 1989; *Proc. Nat. Acad. Sci. USA 86*:3833, 1989). In addition to resolving the problem of immunogenicity, it is also important for the biological activity of vitamin B12 receptor antagonists to select heavy chain constant regions which will impart long serum half life. When using a competitive antagonist, not only its affinity but also the length of exposure to target cells is of critical importance to efficacy. To optionally deplete cells of vitamin B12, blockade of receptors should be maintained for several hours to several days. Human IgG antibodies have half-lives varying from 24 hours to several days. Chimeric IgG antibodies need to be assessed for this property individually. Human IgM have half lives that can exceed several days and, despite their slow rate of penetrance into tissues, may be the most suited to applications where receptor antagonism or modulation must be maintained for prolonged periods.

Completely human monoclonal antibodies can also be created by *in vitro* immunization procedures, employing the specific sorbents used in murine hybridoma generation. *See* Example 1 below and Figure 1. Furthermore, a variety of techniques exist for *in vitro* immunization and human antibody generation (*J. Immunol. Methods 145*:71, 1991; *Hybridoma 9*:81, 1990; *Proc. Nat. Acad. Sci. USA 85*:3995, 1988; *Immunol. Lett. 17*:29, 1988; *BBRC 148*:941, 1987; *Immunol. Lett. 16*:75, 1987; *Tissue Antigens 30*:25, 1987). *See also* U.S. Patent No. 4,879,225 .

In addition to chimeric antibodies, genetic engineering techniques can be used to produce various antibody derivatives including fragments, peptides, or organic molecule mimetics, as well as a variety of antibodies modified with regard to affinity or effector functions. All these various antibody derivatives can be produced from an existing murine antibody to the B-12/TcII receptor. Essentially, one can consider such an antibody as containing, within its antigen combining site, the information necessary to combine with its target and elicit a biologic response. This information can be put into the context of molecules of different size and different forms, and are collectively referred to herein as "antibody derivatives".

The aforementioned chimeric antibodies (which are typically whole IgG) can be engineered by a number of different approaches but essentially seek to

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replace murine constant regions with those of human origin. Alternatively, the CDRs (i.e., the specific regions interacting with the antigen) can be isolated from the antigen combining site and then engrafted into a framework of human variable, as well as constant, regions. This latter type of antibody should be less immunogenic than chimeric antibodies in which only constant regions are replaced. More recently, efforts have been initiated in identifying the most probable residues within a murine antibody structure that elicits antiglobulin or HAMA responses. Essentially, these may be hydrophilic residues that are in contact with solvent and can be identified and replaced by mutagenesis of antibody genes.

For certain applications, it may be favorable to shorten serum half-life in order to provide better penetration into tissues or to clear background blood levels. This can be accomplished by engineering a whole antibody into one of the various fragments identified in Table 2. The most common antibody fragments produced by genetic engineering are the Fab or Fv fragments. Fab fragments can be created by enzymatic digestion of whole IgG, but this usually entails a significant loss in product, as well as inconsistencies in the final antibody form. Thus, genetically engineered Fab is believed to be a more consistent product, and can be produced in gram-per-liter quantities in bacterial expression systems. An important step in producing such engineered fragments is to isolate the regions of the antibody involved in antigen binding (i.e., the CDRs) and place them within the context of human framework. Essentially, Fv is created in a similar manner to Fab, except that the Ch1 and Vh domains are not cloned along with CDR regions. This gives rise to a smaller fragment that requires peptide linkers to join the heavy and light chain components. Moreover, it is believed that certain heavy chain domains can combine with target antigens without the participation of a light chain domain. This is likely to be confined to rather primitive antibodies and antigen-binding specificities. The smallest antibody fragment consists of peptides derived from the information in the CDR, but retain the ability to bind to target structures. Since the affinity of these antibody fragments (as well as Fab and Fv) must be maintained with cloning, bivalent antibody fragments may be created, as well as ones in which mutagenesis and selection has been applied to select a higher affinity version.

Table 2

Genetically Engineered, Antibody Derivatives
Which May Function As Receptor Antagonists

Derivative	References
Antibody Fragments	
CH_2 deletion	Mueller et al., *PNAS 87*:5702-5, 1990; Kashmiri, 3rd IBC Conference on Antibody Engineering, May 14, 1992
CH_3 deletion	Kashmiri, 3rd IBC Conference on Antibody Engineering, May 14, 1992
Fab	Ward et al., *Nature 341*:544-6, 1989; Chiswell & McCafferty, *TIBTech 10*:80-84, 1992; Carter et al., *Biotechnology 10*:163-167, 1988; Better et al., *Science 240*:1041-43, 1988
Fv	Huston et al., *Methods in Enzymology 203*:46-88, 1991; Colcher et al., *JNCI 82*:1191-97, 1990; Skerra & Pluckthun, *Science 240*:10-38, 1988; Whitlow & Filpula, *Methods: A Companion to Methods in Enzymology 2*:97-105, 1991
Heavy chain domain	Ward et al., *Nature 341*:484-5, 1989
MRU/Peptide mimetics	Williams et al., *PNAS 86*:5537-41, 1989; Taub et al., *J. Biol. Chem. 264*:259-65, 1989
Chimeric Antibodies	
Chimeric (mouse V region/ human constant regions)	R.F. Kelley, 3rd IBC Conference on Antibody Engineering, May 14, 1992; Morrison & Oi, *Adv. Immunol. 44*:65-92, 1989; Larrick & Fry, *Hybridoma 2*:172-89, 1991
Primatized (mouse V region/ primate constant regions)	R.A. Newman, 3rd IBC Conference on Antibody Engineering, May 14, 1992
CDR grafted (mouse CDR, human constant and framework regions)	Chiswell & McCafferty, *TIBTech 10*:80-84, 1992; T. Rees, 3rd IBC Conference on Antibody Engineering, May 14, 1992; C. Queen, 3rd IBC Conference on Antibody Engineering, May 14, 1992; Junghans et al., *Cancer Res. 50*:1495-1502, 1990; Tempest et al., *Biotechnology 9*:266-71, 1991; Jones et al., *Nature 321*:522-5, 1986
Hydrophillic residue substitution	T. Rees, 3rd IBC Conference on Antibody Engineering, May 14, 1992
Modified Antibodies	
Antigen Affinity	Ashkenazi et al., *PNAS 87*:7150-4, 1990; Clarkson et al., *Nature 352*:624-628, 1991; Queen et al., *PNAS 86*:10029-33, 1989; Tempest et al., *Bio/Technology 9*:266-72, 1991; Chiswell & McCafferty, *TIBTech 10*:80-84, 1992; Foote & Winter, *J. Mol. Biol. 224*:487-99, 1992
Effector Functions	Wawrzynczak et al., *Mol. Immunol. 29*:213-20, 1992; Wawrzynczak et al., *Mol. Immunol. 29*:221-7, 1992; Lund et al., *J. Immunol. 147*:2657-62, 1991; Duncan et al., *Nature 332*:563-4, 1988; Duncan & Winter, *Nature 332*:738-40, 1988

Bi-specific	Berg et al., *PNAS 88*:4723-7, 1991; D. Segal, *Chem. Immunol. 47*:179-213, 1989; Rodriques et al., *Int. J. Cancer Sup. 7*:1-6, 1992
Di-/multi-meric	Pack & Pluckthun, *Biochem. 31*:1579-84, 1992; H.V. Raff, 3rd IBC Conference on Antibody Engineering, May 14, 1992; M. Whitlow, 3rd IBC Conference on Antibody Engineering, May 14, 1992; Carter et al., *Bio/Immunol. 149*:120-6, 1992
Organic molecule mimetics (peptiomimetic)	Satagovi et al., *Science 253*:792-5, 1991; Wolf, 3rd IBC Conference on Antibody Engineering, May 14, 1992
Immunoadhesins	Marstets et al., *J. Biol. Chem. 267*:5747-50, 1992; Chatnow, et al., *Int. J. Cancer (Suppl.) 7*:69-72, 1992
Anti-idiotypic antibody	Escobar et al., *Viral Immunology 5*:71-79, 1992

Retaining high affinity of an antigen-combining site for its target structure is important for a receptor antagonist since its effectiveness is determined by its binding affinity (in combination with half-life). Numerous techniques have been developed that allow one to increase affinity 2-3 fold (and sometimes up to 5-fold) over native antibody. In addition, as indicated in Table 2, one may modify effector functions either enhancing or (more likely) decreasing complement-activating ability, or the ability to interact with effector cells. Effector functions of a whole antibody used as a receptor antagonist may degrade the selectivity of the receptor antagonist and give more potential for toxicity.

Multi-meric or di-meric forms of antibody fragments may provide advantages from the standpoint of affinity or effector function. Post-translational techniques are known which allow non-covalent association of monomeric antibody derivatives into di- or multi-meric forms leading to enhanced affinity. Such di- or multi-meric molecules may be also more efficient in modulating receptors from surfaces.

As discussed in greater detail below, small molecule receptor antagonists are believed to be more useful for certain medical applications due to their low cost, their utility in oral administration and ease of manufacturing. In addition to peptide structures derived from antibodies (molecular recognition units), molecular modeling techniques can be used to create organic molecule mimetics from antibodies using peptide mimetic intermediaries.

Isolation and cloning of the B-12/TcII receptor allows the creation of a soluble receptor as a competitor. However, such receptor forms may have a short serum half-life, and poor bio-availability. One way to increase the half-life and bio-availability of these cloned receptors is to attach them by recombinant

techniques to immunoglobulin-constant regions. This provides for the longer serum half-life and potential effector functions that may be useful in activity of the receptor antagonist. Such combinations of receptor and immunoglobulin heavy and light chain constant region genes is termed an immunoadhesion.

Using the information present in the combining site of a functional antibody to the B-12/TcII receptor, a second antibody recognizing the combining site of the first can be generated (termed an anti-idiotypic antibody). Such an antibody is the mirror image of the first and thereby an analogue to the receptor itself. Thus, it can be utilized in a manner similar to that of immunoadhesion.

An important step in generating the antibody derivatives discussed above is the isolation of the genes encoding the complementary determining regions of an antibody to the B-12/TcII receptor. This can be accomplished by a number of techniques. For example, a suitable technique involves immunization of mice, hybridoma formation and selection to produce a murine antibody of appropriate specificity. Once murine antibodies are produced, its CDR can be isolated and employed in one of the antibody derivatives identified in Table 2. To elicit such an antibody, the immunogen strategy outlined in Example 1 may be utilized. A similar immunogen approach can be employed in an *in vitro* immunization scheme where antibodies specific for the B-12/TcII receptor can be elicited and then immortalized through EBV infection, electrofusion or hybrid-hybridoma formation. Alternatively, the genes can be isolated through PCR amplification and then cloned into one of the antibody derivatives previously mentioned.

A human antibody can be produced directly from a specific antibody-producing B-cell from a patient having B-cells sensitized to the B-12/transcobalamin II receptor. In such a case, the human-antibody producing B-cell is identified and immortalized either through a cellular technique or through a gene amplification technique. A source for such a B-cell are patients with pernicious anemia, a congenital abnormality associated with low B-12 levels.

In addition, human antibodies may be generated using mice that are transgenic for human immunoglobulin genes. This is accomplished genetically by inserting human IgG genes into the germ line of mice (N. Lonberg, *First Annual Meeting on Commercializing human Monoclonal Antibodies*, December 17-18, 1992). Alternatively, severe combined immunodeficient (SCID) mice have been transplanted with human leukocytes which will proliferate in the mouse and be available for immunization. (Duchosal et al., *Nature 355*:258-262, 1992). In this case, the antibody specific for the B-12/TcII receptor can be isolated after immunization of the human leukocytes, with an immunogen which enhances the

frequency of elicitation of specific antibodies (as described in Example 1 below). Alternatively, antibodies of the appropriate specificity may be generated from combinatorial libraries of germ line IgG genes. In such a process, libraries of Fab fragments, for example, are screened for binding to antigens of the appropriate specificity (essentially equivalent to an un-immunized B-cell repertoire). The same libraries can be created from an immunized repertoire, thus increasing the opportunity for identifying an appropriate antibody. Moreover, the probability of identifying an antibody of appropriate specificity and/or affinity using this technique would be enhanced if prior immunization is carried out following the techniques disclosed in Example 1 below.

Despite the potential utility of antibodies and antibody derivatives as receptor antagonists, there may be pharmaceutical applications for which they are not appropriate due to their cost, potential for immunogenicity, or need for specialized forms of delivery such as orthotopic or oral administration. For these purposes, small organic compounds or peptides may also be developed. Such peptides and compounds may be developed through: (1) screening of bacterial peptide expression libraries, antibody paratope analogs or antibody Fab expression libraries to identify peptide or antibody variable region inhibitors (*Gene 73*:305, 1988; *Proc. Nat. Acad. Sci. USA 87*:6378, 1990; *BioChromatography 5*:22, 1990; *Protein Engineering 3*:641, 1989); (2) rational drug design programs using antibodies as a "pharmacophore" to create organic molecule analogs (*Biotechnology*, Jan. 19, 1991), or traditional rational drug design programs using crystallized vitamin receptor to identify peptide or organic inhibitors (*Biochem. J. 268*:249, 1990; *Science 248*:1544, 1990); and (3) screening a library of organic molecules, as present in fermentation broths of microorganisms, for inhibition of vitamin B12 uptake, identifying the biochemical nature of inhibitory compound(s), and chemically synthesizing analogs to explore structure-function relationship and to identify potent inhibitor(s).

Small organic compounds and peptide receptor antagonists for the B12/TcII receptor may be identified through the use of an appropriate assay. In one embodiment, this assay entails the uptake of radio-labeled vitamin B12, complexed with its carrier protein, transcobalamin II. (*See* Examples 1 and 2 below). Other assays can also prove useful, including specific binding assays using antibodies which act as competitive antagonists. Through these means a repertoire of protein and non-protein molecules suitable for human use can be generated, and may be used to define optimal regimens to manipulate B12 uptake and

bioavailability for different pharmaceutical applications that require an alteration in cellular proliferation.

The manner in which the antibody-based products of the present invention are used is dependent on the mechanism of action of the anti-receptor antibodies and their serum half-life. In one embodiment, the antibody acts as an antagonist of the binding of the complex of vitamin B12/transcobalamin II in a typical mass action fashion. The goal for patient administration is to achieve and maintain serum concentrations of the antibody at a level sufficient to block ≥50%, and more preferably ≥90% or all of the uptake of vitamin B12 into target cells for a prescribed period of time, typically 1 to 7 days. The duration of the blockade is determined by the target cell and the biologic response to be elicited (*e.g.*, cell death or cessation of cell division). One can determine experimentally the degree of inhibition of vitamin B12 uptake by a number of means. If the target cell is one that is easily accessible (*e.g.*, lymphocytes or bone marrow), then samples from patients can be assessed for residual vitamin B12 uptake at various periods following antibody administration. Alternatively, patient samples can be assessed for binding with FITC conjugated anti-receptor antibody using flow cytometry. If it is difficult to acquire patient samples (as in the treatment of solid tumors), an indirect assessment of receptor blockade may be performed by measuring serum levels of antibody using specific immunoassays (*e.g.*, the use of individually specific anti-idiotypic antiglobulin to measure circulating levels of vitamin B12 receptor antibody or other assays as disclosed below) and referring to amounts of antibody required to maintain receptor blockade *in vitro*.

The amount of anti-receptor antibody and timing of administration may also be determined by measuring serum concentrations, as illustrated in Figure 2. For example, antibody administered in a dose range of 1 to 500 mg is quantitated in serum by solid phase, competition ELISA using biotinylated anti-receptor antibody binding to a solid-phase receptor source (*e.g.*, glycoprotein isolate from detergent extract of K562 leukemic cells). Unlabeled antibody is used as a competitor to develop a standard curve. As shown in Figure 2, the serum half-life of a typical IgG antibody is 24 hours, requiring dosing approximately every 36 to 48 hours to maintain serum concentrations above that required for maximal blockade (shown by tri-partite line). The longer the inherent serum half-life of the anti-receptor antibody, the fewer administrations required. Thus, an IgM antibody with a half-life of several days may be more advantageous under certain circumstances.

Antibodies capable of modulating or "capping" vitamin B12 receptors may be used in a manner similar to competitive antagonists. However, knowledge of the parameters of receptor modulation is necessary to optimize therapy. Modulation, capping, patching, clustering, or immobilization can be the result of interactions of antibodies with cell surface antigens or receptors. The terms describe a range of responses from complete clearance of antigen from the surface to an inhibition of antigen mobility within the membrane. Whatever type of interaction occurs, antibody binding can result in a loss of function or triggering of a biological response, depending on the nature of the antigen. There have been a variety of antigens and receptors demonstrated to undergo modulation when bound by antibody, but there appears to be little relationship between antigen number and the ability to be modulated (*Acta Haemotol. 73*:196, 1985). Since bivalency (or multi-valency) is required to cross-link antigen and cause modulation, the ability to do so is governed by antigen density and distribution, as well as the distance spanned by antibody (*e.g.*, an IgM can span a greater distance than IgG). In addition, accessory antigens, antibodies or cells can also enhance modulation. For instance, modulation of receptors is enhanced by the presence of complement, by HIV gp120 protein for CD-4 on T-cells, and by monocytes for CD-5 modulation on T-cells (*J. Immunol. 133*:2270, 1984; *Science 245*:1380, 1989; *J. Immunol. 144*:239, 1990).

Essential for modulation is the epitope on the target antigen, recognized by antibody. *J. Immunol. 137*:2286, 1986. In the case of cell surface IgD immunoglobulin on B-cells, antibodies modulate according to the portion of the cell surface IgD molecule bound. *J. Immunol. 139*:2873, 1987. Once modulated, antigen or receptor can have several fates: immobilization or clustering on the cell surface, internalization and degradation or shedding. The degree of modulation may vary significantly even with the same antibody, antigen, and target cell population. *Acta Haematol. 76*:119, 1986. Whichever fate, biological responses may be suppressed or triggered by modulation and not be reestablished for periods of 24 to 72 hours, coincident with antigen or receptor re-expression.

Selectivity may be achieved in modulation also. Most types of cells can be modulated by antibodies as described for fibroblasts (*J. Cell Sci. 98*:191, 1991), adipocytes (*Int. J. Immunopharmacol. 6*:193, 1984), pancreatic islet cells (*Diabetologia 24*:117, 1983), sperm (*Exp. Cell Res. 144*:275, 1983), glomerular epithelium (*J. Immunol. 135*:2409, 1985) and tumor cells (*Int. J. Cancer 448*:1095, 1989). However, modulation is most readily achieved on lymphoid cells. Depending on the tissue location, such lymphocytes may be more or less

susceptible to modulation. For instance, antibody to OKT-3 like antigens on guinea pig T-cells were susceptible to modulation when present in all lymphoid tissues except thymus. *J. Immunol. 138*:2500, 1987. CD-5 antigen or human T-cells can be modulated on peripheral cells without modulation of T-cells in lymph nodes by controlling the dose of antibody. The reverse can also be achieved by injecting a modulating dose of T101 (anti-CD-5) for peripheral cells, and following with a subsequent second infusion of T101 which is delivered selectively to lymph node T-cells (*J. Immunol. 133*:1641, 1984; *N. Eng. J. Med. 315*:673, 1986). Modulation is not restricted to just antibodies; small compounds and peptides can also cause redistribution of a receptor (*J. Biol. Chem. 167*:3530, 1992).

Common to many non-neoplastic disease processes is a stage in which the disease process itself, or its symptoms, can be halted or ameliorated by the use of an anti-proliferative agent such as vitamin B12 receptor antagonists. These commonly recognized stages include a sensitization or elicitation phase in which immune cells responsible for the disease become turned on by antigen specific or non-specific means, followed by a proliferative phase in which the immune cells expand in number, and finally a symptomatic phase in which the expanded immune cells create tissue damage directly or indirectly. Because of this, anti-proliferative chemotherapeutic drugs are commonly utilized in the treatment of many diseases other than cancer, but are limited in use to life threatening situations due to their associated toxicity. Anti-proliferative agents, such as the ones of the present invention (with little of the direct toxicity of chemotherapeutic drugs), may be used more widely. More specifically, the anti-receptor agents of the present invention are not destructive to plasma membrane processes (e.g., ion transport). In addition, the anti-proliferative activity is reversible by administration of vitamin B12. Furthermore, the agents of this invention may not be mutagenic, teratogenic, or carcinogenic since they act at the level of the plasma membrane, and not at the level of the nucleus, and DNA by intercalation or cross-linking (as many chemotherapeutic drugs act).

An understanding of the pharmaceutical applications for B12/TcII receptor antagonists requires a knowledge of the cell types targeted by such therapy. To this end, various pharmaceutical applications are disclosed in Table 3 below.

Table 3

Target Cells for Vitamin B12 Receptor Antagonists

Target Cell	Other Proliferation Associated Markers	Potential Pharmaceutical Applications
Activated T-Cell	IL-2 receptor Transferrin Receptor Insulin Receptor Class II Histocompatibility Antigens	Graft versus Host Disease Organ Transplants Auto-Immune Diseases Asthma Crohn's Disease
Tumor Cells	Tumor Assoc. Ags. Ki67 Transferrin Receptor	Tumor Therapy (alone and in combination with chemotherapeutic drugs)
Bone Marrow Stem Cells	CD-34 Transferrin Receptor Class II Histocompatibility Antigens IL-1, IL-3 Receptors	Allogeneic Bone Marrow Transplants Reduction in Toxicity of Chemotherapy
Proliferating Fibroblasts	Thy 1.1 Transferrin Receptor Insulin & Insulin-like Growth-Factor Receptors Fibroblast Growth-Factor Receptor	Inhibition of Adhesions, Scarring Scleroderma
Proliferating Epithelium or Epidermal (Keratinocytes)	EGF Receptor Proto-Oncogenes	Psorasis

Proliferating and activated T-cells can cause a wide variety of diseases ranging from the chronic inflammation of Crohn's disease to more acute organ graft rejection. In all of these diseases, the T-cell may serve a central pathogenic role or a more accessory role. Anti-proliferative chemotherapeutic drugs serve to reduce symptomotology and in some cases lead to long-term remission. Similarly, proliferating fibroblasts and epithelial cells may give rise to diseases characterized by cell overgrowth. Vitamin B12 receptor agents may be

used to replace or used in combination with existing chemotherapeutic regimens in these diseases. An important aspect of the use of anti-proliferative vitamin B12 receptor agents in these diseases is not to apply it so aggressively or with improper timing such that normal healing (adhesions, scarring) or cell renewal (psorasis) processes are also inhibited. As such, low doses of anti-receptor agents may be used during healing and higher doses once healing is completed. Alternatively, anti-receptor agents may not be administered at all until after healing is completed.

As previously mentioned, B12/TcII receptor antagonists can be used to deprive neoplastic cells of vitamin B12. It has already been shown that sufficient deprivation leads to the death of rapidly proliferating lymphoid neoplasms such as leukemia and lymphoma. Moreover, short term treatment to reduce cellular availability of this nutrient, combined with existing chemotherapeutic agents, markedly improves therapeutic efficacy.

For solid tumors, B12 depletion may induce cytostasis and differentiation as well as cell death. Thus, B12/TcII receptor antagonists may be used to induce differentiation in hormonally responsive solid tumors. An increase in the number of cells expressing a differentiated phenotype should translate into an increase in expression of hormone receptors. The hormone receptor status of tumors, such as breast and prostrate cancer, are directly correlated with their response to hormonal therapy. Accordingly, B12/TcII receptor antagonists can be used to increase the number of receptor positive tumor cells or increase receptor density in order to enhance efficacy of subsequent hormonal therapy.

Vitamin B12 receptor antagonists may affect both replicating neoplastic and normal cells. However, bone marrow progenitors demonstrate differential sensitivity or response. Thus, B12 receptor antagonists can be used to modulate sensitivity of bone marrow progenitors so as to enhance their resistance to the toxic effects of chemotherapeutic agents. Such chemotherapeutic drugs act primarily on replicating cells, with non-replicating cells being much less sensitive. Antibodies are well suited for this application since delivery is more readily achieved to highly accessible marrow versus normal organs and solid tumors. In addition, a B12/TcII anti-receptor antibody, possessing the ability to modulate receptor, could differentially effect lymphoid versus epithelial tissues. Decreasing the sensitivity of progenitors to toxic drugs would increase the bone marrow reserves and enhance subsequent response to colony stimulating factors, and enable higher doses of chemotherapy or reduce the interval to reconstitution. It should also be recognized that such positive effects on bone marrow progenitors, as a natural consequence of B12 receptor therapy for cancer, is an additional mechanism

by which the therapeutic index of chemotherapeutic drugs other than 5-FU and methotrexate can be improved.

In a variety of autoimmune diseases, graft versus host disease, ectopic allergy, and organ transplantation, an initial `induction' phase, in which the patient becomes sensitized to self or allo-antigens, is followed by a "proliferative" phase in which forbidden or unregulated clones of B-or T-cells are expanded. It has long been known that treatment with anti-proliferative, chemotherapeutic drugs following induction can inhibit expansion of forbidden clones, inhibit progression of disease, and restore a stable state of tolerance. An antibody, OKT-3, that controls the proliferation of allo-antigen-sensitized T-cells, has been approved for management of acute allograft rejection. Anti-receptor antibodies of the present invention can be substituted for extremely toxic chemotherapeutic drugs or highly immunogenic antibodies such as OKT-3 and achieve a similar state of tolerance without these associated drawbacks.

Inflammation is an application for which antibodies are already being utilized in clinical trials. The primary emphasis has been on inhibiting the early manifestations of inflammation by inhibiting recruitment or binding of inflammatory cells to vascular endothelium of injured tissue. It also well recognized that proliferation of cells at the site of inflammation contributes to the pathology and tissue destruction of both acute as well as chronic inflammation. To this end, anti-proliferative, chemotherapeutic drugs have been widely used to inhibit sequelae of inflammation.

Methotrexate is one such drug commonly used to treat symptoms associated with rheumatoid arthritis. The drug acts to reduce both localized (e.g., synovium) and generalized inflammation associated with disease progression. Methotrexate acts synergistically with vitamin B12 depletion in therapy of leukemia. B12 antagonists can therefore be combined with methotrexate to enhance efficacy in rheumatoid arthritis. Other methotrexate applications include treating destructive inflammation associated with chronic heart disease and colitis.

Surgery, radiation or chemotherapy to the abdomen is often complicated by the development of tissue adhesions. These represent a considerable clinical problem because they lead to bowel blockage and require surgical intervention. Peritoneal adhesions arise as a result of proliferation of the cells of the peritoneal membrane lining the abdomen. A non-toxic means of interfering with such proliferation could lead to restoration of these normal cells to homeostatic control mechanisms and thereby inhibition of adhesion formation. A similar process of benign proliferation and subsequent scarring is a complication of

retinal surgery. Direct instillation of a small molecule analog of an antibody receptor antagonist could prevent such disabling complications.

The following examples are designed to illustrate the production and pharmaceutical use of certain vitamin B12/TcII receptor antagonists and modulators. The type of receptor antagonist and modulator used in the examples is a human or chimeric antibody applied to the treatment of AIDS Related Lymphoma (ARL), a particularly aggressive form of cancer arising in AIDS patients, as well as other medical applications. Small molecule and peptide analogs may also be used for treatment of cancer, but are more optimally used in other pharmaceutical applications. The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

Identification and Characterization of Functional Monoclonal Antibodies to the Vitamin B12 Receptor

Hybridomas are generated by PEG mediated fusion of murine splenocytes from mice, immunized as shown in Figure 1, and HGPRT- myeloma cells like NS-1. For immunogens, transcobalamin II, present in Cohn-purified serum protein, is covalently immobilized (CnBr Sepharose)* and used to adsorb small quantities of solubilized receptor. The complex is then used to immunize mice. Four to six weeks after fusion, hybridoma supernatants are screened in a functional assay for inhibition of vitamin B12 uptake in K562 leukemic cells cultured in chemically defined medium using a modified radiolabeled assay with 57 Co-cobalamin complexed with transcobalamin II from Cohn fractions. The results of the primary screen in microtiter plates are illustrated in Table 4 below and expressed as the fraction of the uninhibited control (well A1). Well H12 serves as the positive control (maximum inhibition) and utilizes serum as a source of unlabeled vitamin B12 complexed to transcobalamin II as competitor.

*Trademark

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Table 4

Primary Screen of Hybridomas

	1	2	3	4	5	6	7	8	9	10	11	12
A	**1.000**	.986	.995	.973	**.322**	.898	.994	.993	.982	.988	.987	1.000
B	.788	.922	.888	.965	.986	.923	.898	.993	.942	.986	.897	.954
C	.972	**>1.000**	.984	.832	.964	.777	.885	.924	.987	.845	.892	1.000
D	.983	**.111**	.986	.799	.912	.943	1.000	.956	.964	.955	.913	.987
E	.788	.922	.888	.965	.986	.923	.898	.993	.942	.986	.897	.954
F	1.000	.986	.995	.973	.988	.898	.994	.993	.982	**.198**	.987	1.000
G	.983	.986	.986	.799	.912	.943	1.000	.956	.964	.955	.913	.987
H	.972	1.000	.984	.832	.964	.777	.885	.924	.987	.845	.892	**.089**

The hybridomas identified in this primary screen (A5, C2, D2, and F10) are cloned by limiting dilution with thymic feeder cells. Four to six weeks later, clones (identified by sequential numbering) from the primary wells are rescreened in the functional assay to identify those clones retaining the characteristic activity of the parents. In addition other assays are performed to characterize the specificity of the clones by inhibition of vitamin B12 uptake on carcinoma versus leukemia cells or normal, mitogen stimulated lymphocytes. The results of the assessment of specificity are shown in Table 5 below.

Table 5

Assessment of Specificity

	Target Cells		
Clone Lymphocytes	Carcinoma	Leukemia	Normal
A5/8	.386	.333	.287
A5/12	.342	.384	.317
C2/2	.989	>1.000	>1.000
C2/5	.923	>1.000	>1.000
D2/20	.656	.089	.154
D2/7	.891	.174	.245
F10/4	.198	.123	.423
F10/8	.234	.312	.666

Based upon these results the antibody D2/20 is selected for further evaluation in treatment of lymphoma. The antibody is able to strongly inhibit

vitamin B12 uptake at levels of antibody as low as 10 nanograms/ml (not shown). In addition, the antibody appears to inhibit uptake of vitamin B12 in lymphoid cells but not those of epithelial origin, a characteristic which is potentially useful in decreasing toxicity to replicating crypt cells in colonic epithelium. In other assessments, the antibody did not inhibit uptake of vitamin B12 in mitogen stimulated murine splenocytes, indicating its specificity for the human receptor.

Example 2

In Vitro Assessment of Cell Killing Potential of Vitamin B12 Anti-Receptor Antibody Alone and In Combination with Chemotherapeutic Drugs

Antibody D2/20 at a range of concentrations is incubated with Raji Burkitt lymphoma cells in microtiter plates for three days with and without chemotherapeutic drugs. Cell viability is measured by reduction of tetrazolium dye. Only viable cells metabolize the dye to an insoluble, colored product which is subsequently solubilized and read in a spectrophotometer. The results of the assay are shown in Table 6 below.

Table 6

	Antibody (nanograms/ml)				
	100	**10**	**1**	**0**	
	.268	.435	.723	.987	**0**
	.055	.077	.212	.993	**0.1**
Methotrexate (micrograms/ml)					
	.048	.052	.089	.798	**1**
	.047	.048	.054	.563	**10**

100% lysis control = .047

Based on these results, the antibody to the vitamin B12 receptor is able to elicit cell death of the lymphoma cells, presumably by starving them of vitamin B12. In addition, when combined with methotrexate, the combination appears to be synergistic since it was considerably more active than either of the

two agents alone. The results are consistent with those obtained with other methods of vitamin B12 depletion.

Example 3

In Vivo Assessment of Vitamin B12 Anti-Receptor Antibody in Combination with Chemotherapeutic Drug

Nu/nu mice are injected subcutaneously with 1 million Raji Burkitt lymphoma cells. After two weeks, barely palpable nodules are present at the injection site. Measurements are made in three dimensions with a planarimeter and equally sized tumors assigned to experimental groups of 10 mice. Mice are injected intravenously with the drug, methotrexate (3 dose levels-50, 10, and 5 milligrams/M^2) and antibody D2/20 at 100 micrograms/mouse. Therapy is administered once weekly. Controls of antibody and drug alone, as well as vehicle controls, are included. Mice are monitored visually for toxicity, death, and tumor size weekly for 8 weeks, at which time the experiment is terminated, the mice sacrificed, and the tumors removed and weighed. The average of serial tumor measurements converted to weight (grams) is shown in Table 7 below.

Table 7

Group	Week 1	2	3	4	5	6	7	8
Vehicle	.05	.12	.34	.67	1.3	2.9	6.0	--
Antibody (Ab)	.06	.09	.12	.22	.35	.57	.83	1.02
Drug (50)	.04	.10	.25	.41	.73	1.4	2.3	3.4
Drug (10)	.05	.12	.30	.53	.92	2.1	4.8	6.9
Drug (5)	.07	.14	.43	.70	1.2	2.6	4.8	7.2
Ab + Drug (50)	.05	.07	.11	.09	N.D.	N.D	N.D.	N.D.
Ab + Drug (10)	.06	.08	.13	.15	.12	.07	N.D.	N.D.
Ab + Drug (5)	.05	.07	.11	.21	.24	.17	.15	.11

(--) = Not measurable due to tumor necrosis
N.D. = Not detectable

Based upon these results, it can be concluded that vitamin B12 anti-receptor antibody is active in inhibiting tumor growth in this model of human Burkitt lymphoma, and that its combination with methotrexate provides a more effective regimen.

Example 4

Treatment of a Patient with AIDS Related Lymphoma with Vitamin B12 Anti-Receptor Antibody in Combination with Chemotherapy

A patient diagnosed with AIDS Related Lymphoma is admitted to the hospital for treatment. The patient presents with CNS involvement and poor prognosis and is suffering from a fever of unknown origin. The patient has CD-4 counts below 200/μl and has been receiving anti-retroviral therapy, AZT* (zidovudine), prior to diagnosis of ARL. The patient is given an aggressive regimen combining chemotherapy with bone marrow support (rGM-CSF) according to the following protocol:

A. Cyclophosphamide,* 200 mg/M², IV over 30 minutes daily on days 1 through 5;

B. Vincristine* 1.4 mg/M² IV push on day 1, not to exceed 2 mg/dose;

C. High dose methotrexate, 1500 mg/M² day 1, 150 mg/M² administered over 30 minutes with the subsequent 1350 mg/M² administered over the next 23 1/2 hours, rapid urine flow maintained with the urine pH's supplemented with sodium bicarbonate to maintain the urine pH >7.5;

D. Folinic acid, 30 mg IV or PO administered q6h, beginning 12 hours after the completion of the methotrexate infusion, folinic acid continued until the serum methotrexate level is .01 uM;

E. Mitoxanthrone,* 10 mg/M2 IV push on days 4 and 5;

F. Decadron,* 5 mg/ M2 IV push on days 4 and 5;

G. rGM-CSF, 3 μg/kg subcutaneously bid, through day 6, until the absolute granulocyte count is > 1,000/ul; and

H. Cytarabine* (50 mg) intrathecal on day 1 of course 1; thereafter intrathecal methotrexate (12 mg) on day 1 and intrathecal cytarabine on day 16 for each of 6 other courses of therapy.

*Trademark

The patient is also administered antibiotics and Diflucan* prophylactically. AZT is discontinued during chemotherapy. The patient receives seven courses of therapy and is assessed to have experienced a partial response of nodal disease and a complete response of CNS disease. After 7 months the patient returns to the hospital with relapsing disease peripherally, but still negative for CNS involvement.

The patient is treated with the same combination regimen with the following exceptions: rGM-CSF is not included due to concerns for accelerating tumor growth, no intrathecal treatment, and the inclusion of vitamin B12 receptor antibody. In particular, antibody is administered on day 1 of each course of chemotherapy. The antibody component of the regimen consists of a "humanized" chimeric IgM derived from the murine antibody D2/20, administered at a dose of 100 mg in a IV drip over 4 hours. The antibody has been previously determined to have a serum half life of 72 hours in patients.

The patient is removed from treatment after only 3 courses due to a non-responding neutophil count due to the lack of rGM-CSF. After 4 months however, the patient is assessed to have experienced a complete response of peripheral disease. The patient continues in complete response for 17 months.

Example 5

Identification of Monoclonal Antibodies Capable of Vitamin B12 Receptor Modulation

The hybridomas positive in the assay for inhibition of Vitamin B12 uptake (Example 1 above) are screened in a different assay designed to identify those hybridomas capable of receptor modulation. Hybridoma supernatants are incubated with 1 x 10^6 K562 leukemic cells at 4°C for 60 minutes. Cells are resuspended, washed and an equal aliquot of cells (5 x 10^5) removed to a separate tube and incubated at 37°C for 60 minutes while the other aliquot is retained at 4°C for the same period. Both aliquots from each hybridoma are analyzed for bound mouse immunoglobulin by staining with fluorescein isothiocyanate-conjugated, anti-mouse immunoglobulin (FITC-αMIg). Unbound, secondary antibody is removed by washing, and stained cells examined using a Coulter Epics C* flow cytometer. The mean fluorescent intensity (MFI) of positive cells and the binding profile are compared on the two aliquots of cells. Of the antibodies identified in

*Trademark

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Example 1, only the sister clones F10/4 and F10/8 are positive for receptor modulation as shown in Figure 3. Fluorescence intensity of the sample held at 37° C is significantly lower than the one held at 4°C, and constitutes preliminary evidence of receptor modulation.

Parameters for receptor modulation or "capping" are further detailed by studies with microtubule and microfilament inhibitors like colchine or vinblastine, to demonstrate the requirement of cytoskeleton in modulation. Studies are also performed with sodium oxide to demonstrate the dependence of capping on cellular energy processes. In addition, the time to complete expression of receptors is determined to be 24 hours, and it is determined that only nanogram/ml levels of antibody are required to maintain cells devoid of receptors which results in complete inhibition of thymidine uptake within 72 hours.

Example 6

Treatment of a Patient with Graft Versus Host Disease (GVHD) With Vitamin B12 Anti-Receptor Antibody

An adult patient with acute leukemia enters the hospital for an induction regimen prior to bone marrow transplantation. The patient receives cytosine arabinoside, 3g/M^2, every 12 hours for 6 days, followed by fractionated total body irradiation, 200 cGy, twice daily for 3 days. The patient is administered T-cell depleted, histocompatible marrow following induction, along with cyclosporine and methotrexate for prophylaxis of GVHD. The cyclosporine is administered through a silastic catheter from day 1 through day 180 at a dose level of 1.5 mg/Kg/d for the first 15 days, followed thereafter at a dose level of 3 mg/Kg/d. Methotrexate is administered at a dose of 0.25 mg/Kg/d on days 1, 3, 6, 11, 18, 25, and 31.

The patient demonstrates engraftment and has no evidence of GVHD up to 3 months. At that time, however, the patient is readmitted to the hospital and diagnosed as suffering from Grade III GVHD while still receiving cyclosporine A. The patient is once again administered methotrexate but in combination with Vitamin B12 anti-receptor antibody. The regimen consists of administration of a "humanized" chimeric IgM derived from the murine antibody F10/4 at a dose of 50 mg in an IV drip over 4 hours followed by methotrexate infusion (0.25 mg/Kg/d). The regimen is administered on days 1, 3, 6, and 11 while maintaining cyclosporine administration. After two weeks most manifestations of GVHD have

resolved and the patient is maintained on cyclosporine for an additional 60 days. The patient remains free of GVHD for two years at which time he relapses from leukemia and dies.

Example 7

Reduction in Hematologic Toxicity of Chemotherapeutic Drugs With Vitamin B12 Anti-Receptor Antibody

A patient with stage IV colon cancer with both lymph node and liver involvement is admitted to the hospital for treatment. The patient is administered a regimen of leucovorin (200 mg/M^2), given as a 10-minute infusion, followed by a dose of 1,000/M^2 of 5-fluorouracil every two weeks. Therapy is stopped after 2 months due to grade III/IV leukopenia and thrombocytopenia. The patient experiences a partial response of liver and lymph node disease and only minimal neurotoxicity.

The patient is re-treated by prior infusion of an IgM, "humanized" chimera of antibody F10/4, capable of modulating the vitamin B12 receptor. The patient is infused with 2 mg of antibody over 2 hours, a dose found previously to modulate the receptor on bone marrow cells, but which is virtually undetectable by immunoperoxidase in biopsies of solid tumor lesions of patients. After 18 hours the patient is infused with 5-fluorouracil and leucovorin as before. The patient continues receiving treatment every 2 weeks for 4 months and experiences only Grade I thrombocytopenia and moderate neurotoxicity. After this second treatment interval, the patient is assessed to have experienced a complete response of lymph node disease with a virtual complete response of liver disease.

While the present invention has been disclosed and described with reference to specific embodiments, it will be understood by those skilled in the art that various changes or modifications in form and detail may be made without departing from the spirit and scope of this invention.

CLAIMS

1. An antibody or antibody derivative to a vitamin B12/transcobalamin II receptor, the antibody being capable of preventing or inhibiting cellular uptake of vitamin B12.

2. The antibody of claim 1 wherein the antibody is a monoclonal antibody.

3. The antibody of claim 1 wherein the antibody is an Fv fragment.

4. The antibody of claim 1 wherein the antibody is D2/20.

5. An antibody or antibody derivative according to any one of claims 1 to 4 for use as an active therapeutic substance.

6. An antibody or antibody derivative according to claim 5 for inhibiting cell division of a target cell in a warm-blooded animal.

7. An antibody or antibody derivative according to claim 5 for preventing or inhibiting cellular uptake of vitamin B12 in a warm-blooded animal.

8. Use of an antibody or antibody derivative according to any one of claims 1 to 4 for the manufacture of a medicament for preventing or inhibiting cellular uptake of vitamin B12 in a warm-blooded animal.

9. Use of an antibody or antibody derivative according to any one of claims 1 to 4 for the manufacture of a medicament for inhibiting cell division of a target cell in a warm-blooded animal.

A

FIG. 1

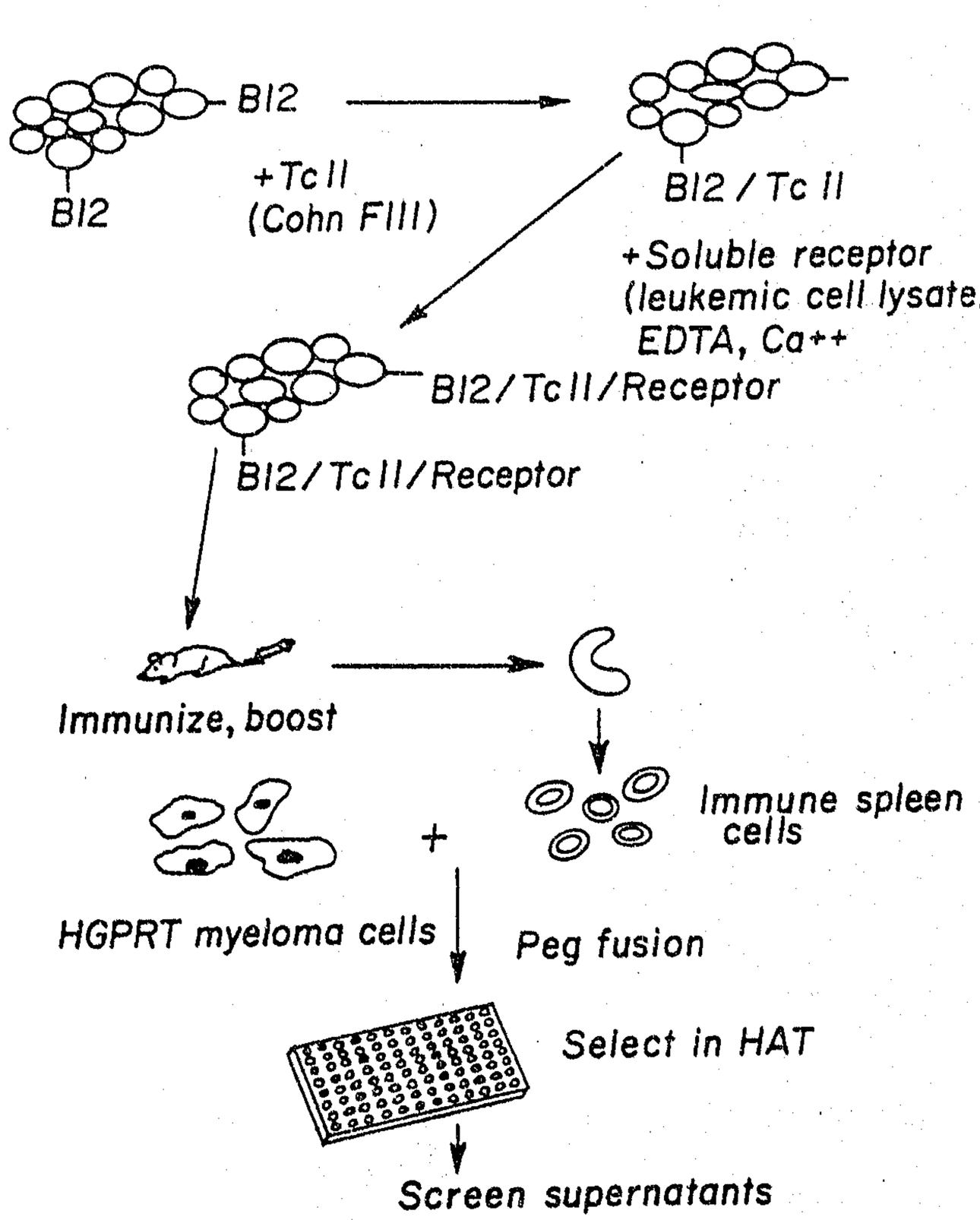
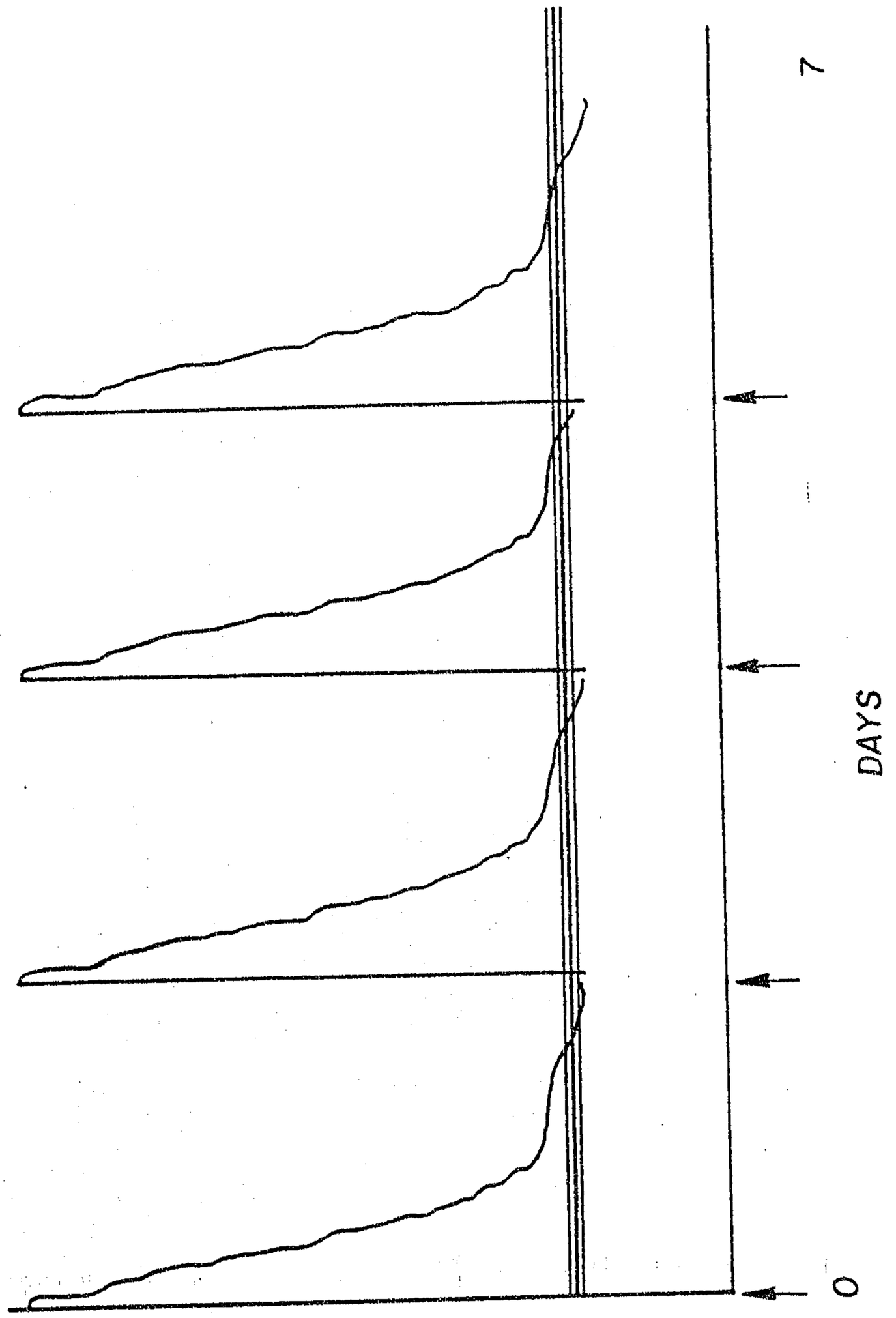
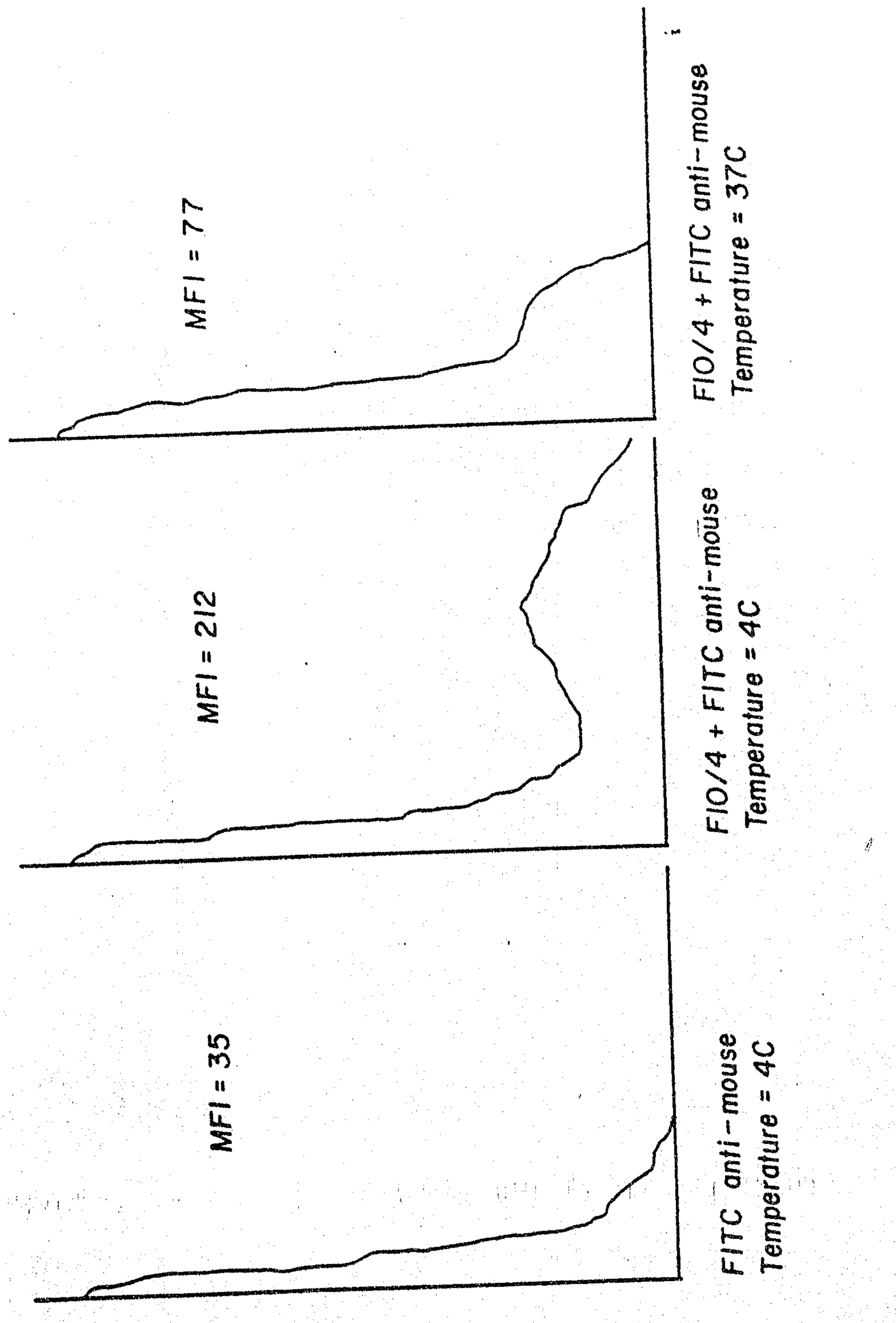

DEVELOPMENT of VITAMIN B12 RECEPTOR ANTIBODIES
B12
B12
+Tc II (Cohn FIII)
B12 / Tc II
+Soluble receptor (leukemic cell lysate) EDTA, Ca++
B12/Tc II/Receptor
B12/Tc II/Receptor
Immunize, boost
Immune spleen cells
HGPRT myeloma cells
Peg fusion
Select in HAT
Screen supernatants

FIG. 2


(ordinate = Anti-receptor antibody levels measured by specific serum immunoassay)
DAYS
0
7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FIO/4 + FITC anti-mouse
Temperature = 37C
MFI = 212
FIO/4 + FITC anti-mouse
Temperature = 4C
MFI = 35
FITC anti-mouse
Temperature = 4C

FIG. 3

DEVELOPMENT of VITAMIN B12 RECEPTOR ANTIBODIES