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(54) **ANTIBODY PREPARATION**

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(76) Inventors: **Herman Waldmann**, Oxford (GB);
Mark Frewin, Oxford (GB)

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Correspondence Address:
Nixon & Vanderhye
Eighth Floor
1100 North Glebe Road
Arlington, VA 22201-4714 (US)

ABSTRACT

(21) Appl. No.: **09/736,371**

An IgG antibody is provided having a binding affinity for the CD3 antigen complex in which in the heavy chain has a variable region framework together with at least one CDR selected from the amino acid sequences of SEQ ID No 2, 4 and 6 and respective conservatively modified variants thereof and the light chain has a variable region framework together with at least one CDR selected from the amino acid sequences of SEQ ID No 8, 10 and 12 and respective conservatively modified variants thereof

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Related U.S. Application Data

characterised in that the heavy chain variable region framework corresponds in sequence to the human type sequence and the light chain variable region framework includes one or more of the specific amino acids characteristic of the rodent type sequence.

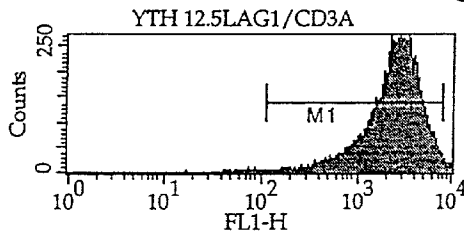
(63) Continuation of application No. PCT/GB99/02380,
filed on Jul. 21, 1999.

(30) **Foreign Application Priority Data**

Jul. 21, 1998 (GB) 9815909.8

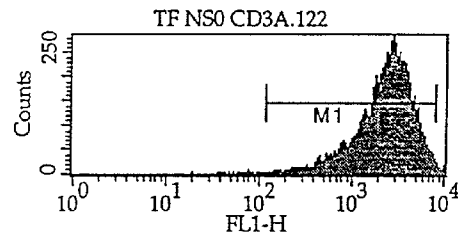
The novel antibody is capable of being expressed by mammalian cell expression systems at enhanced yields.

Fig.1.



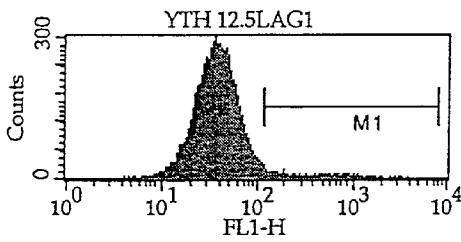
File: YTH 12.5LAG1/CD3A

Marker	Left, Right	% Gated	Median
All	1, 9647	100.00	2458.24
M1	111, 7774	98.60	2458.24



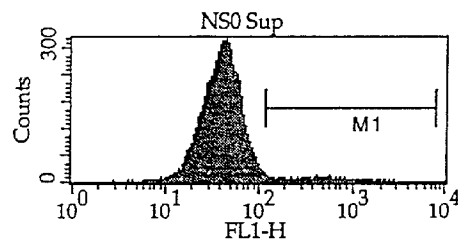
File: TF NS0 CD3A.122

Marker	Left, Right	% Gated	Median
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M1	111, 7774	98.78	2287.57



File: YTH 12.5LAG1

Marker	Left, Right	% Gated	Median
All	1, 9647	100.00	37.86
M1	111, 7774	4.66	259.46



File: NS0 Sup

Marker	Left, Right	% Gated	Median
All	1, 9647	100.00	39.24
M1	111, 7774	3.68	378.55

YTH 12.5LAG1/CD3A.27 = rat anti human CD3 light chain + humanised CD3A heavy chain

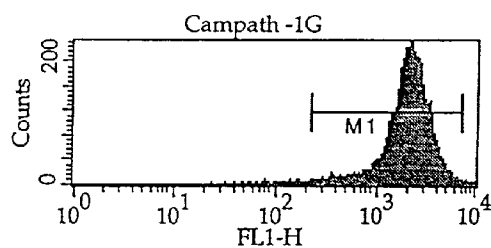
TF NS0 CD3A.122 = humanised CD3A

YTH 12.5LAg1 = anti human CD3 light chain only

NS0 = Transfection line for humanised CD3

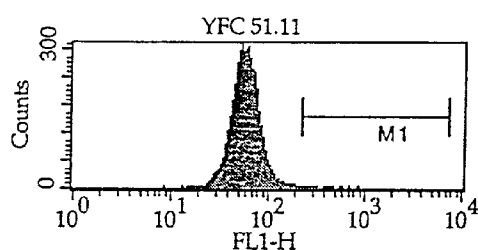
Fig.2.

TF 12.5L/CD3A.27



File: Campath -1G

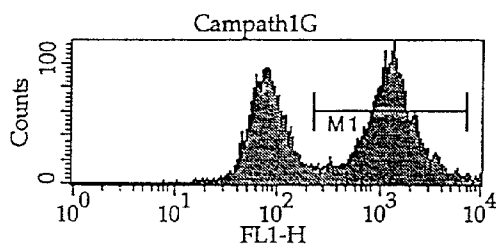
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M1	221, 6978	98.56	1980.96



File: YFC 51.11

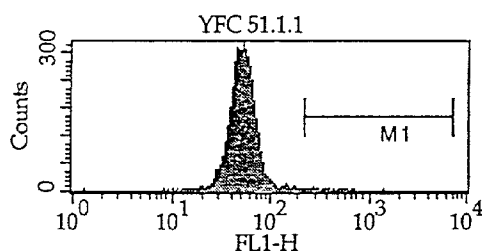
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M1	221, 6978	0.56	299.61

TF 12.5L/CD3A.34



File: Campath1G

Marker	Left, Right	% Gated	Median
All	1, 9647	100.00	673.17
M1	221, 6978	58.70	1197.09



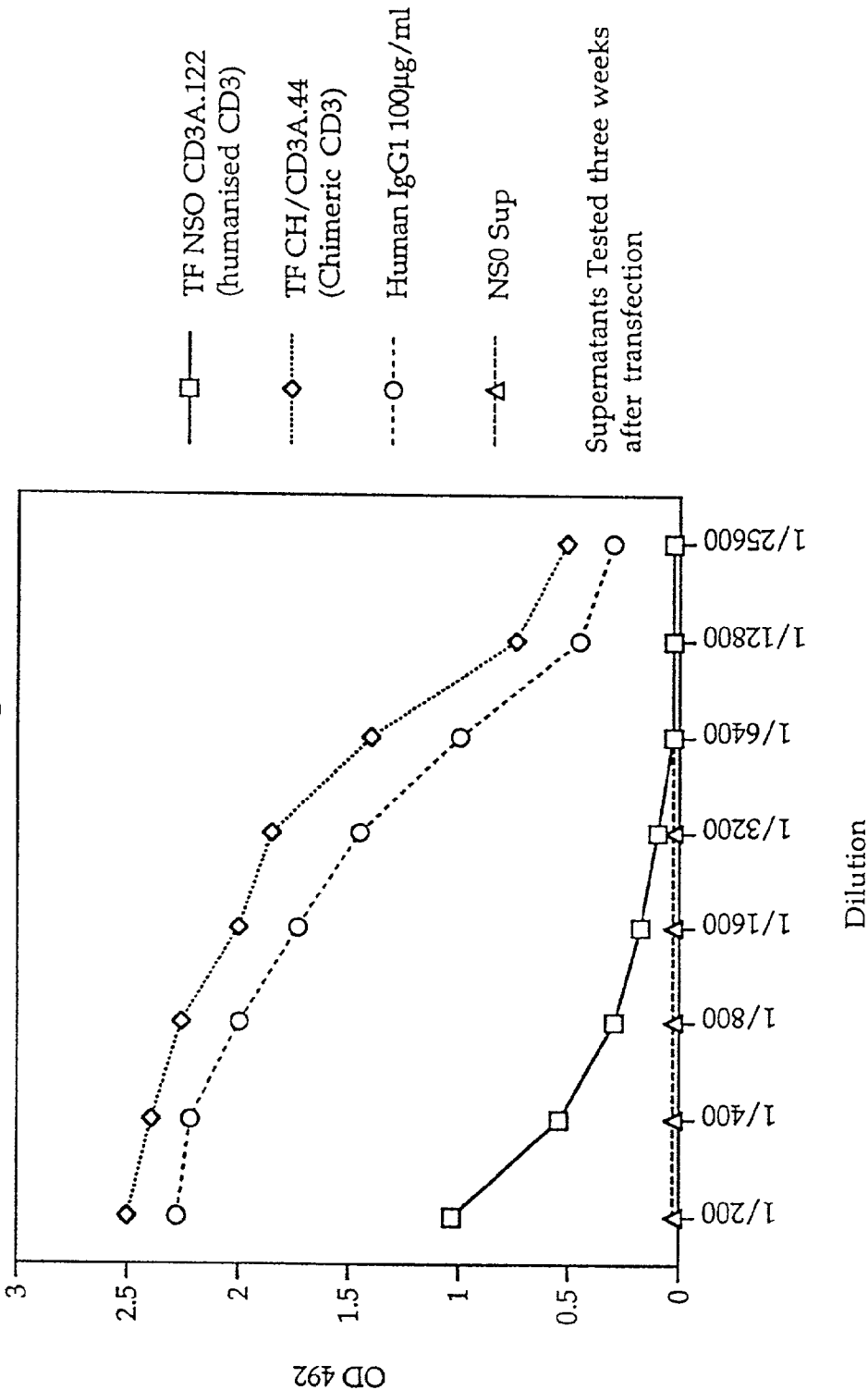
File: YFC 51.1.1

Marker	Left, Right	% Gated	Median
All	1, 9647	100.00	50.48
M1	221, 6978	0.73	339.82

Campath-1G = rat anti human CD52 IgG2b

YFC 51.1.1 = rat anti human CD18 IgG2b isotype control

Fig.3.



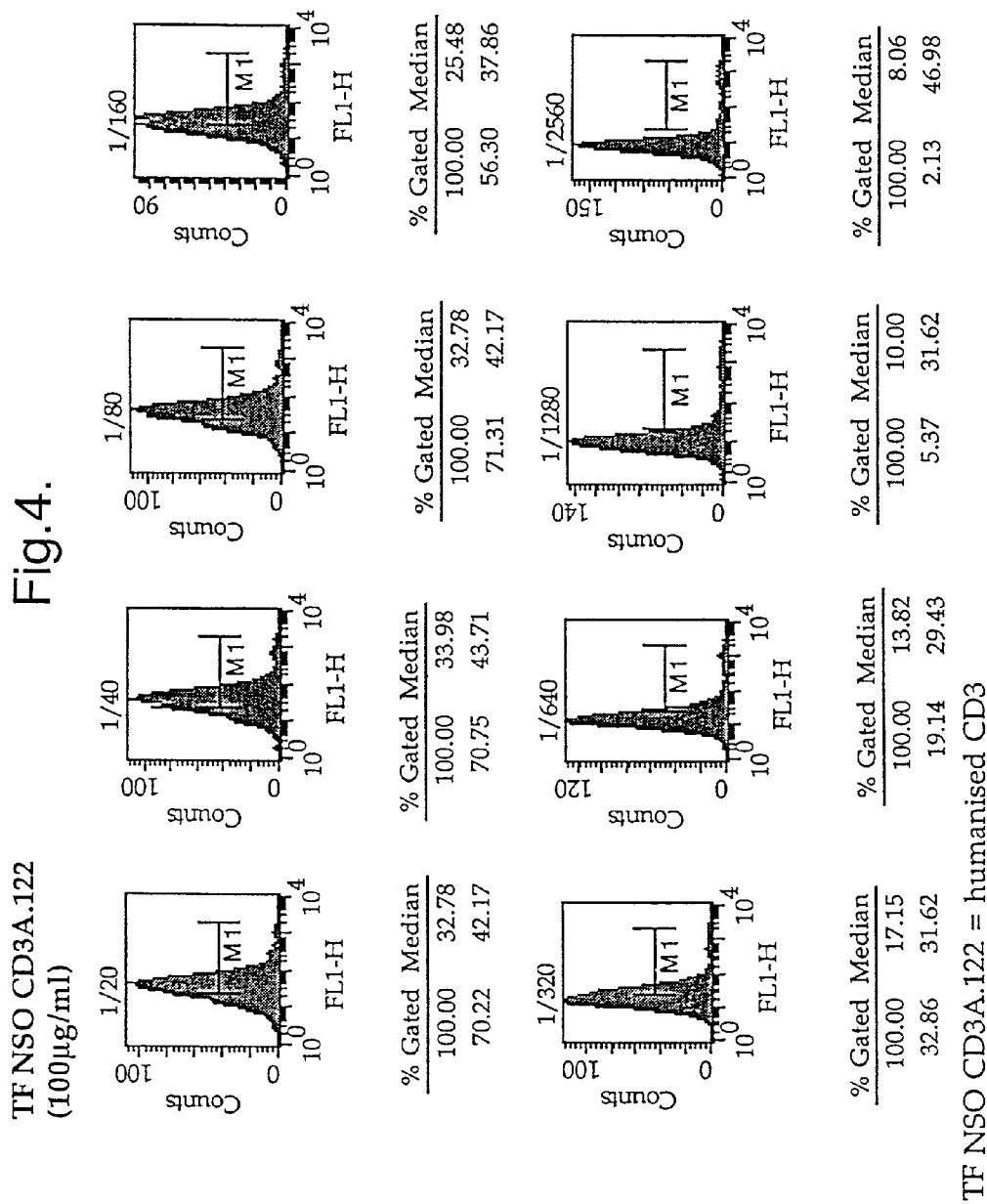
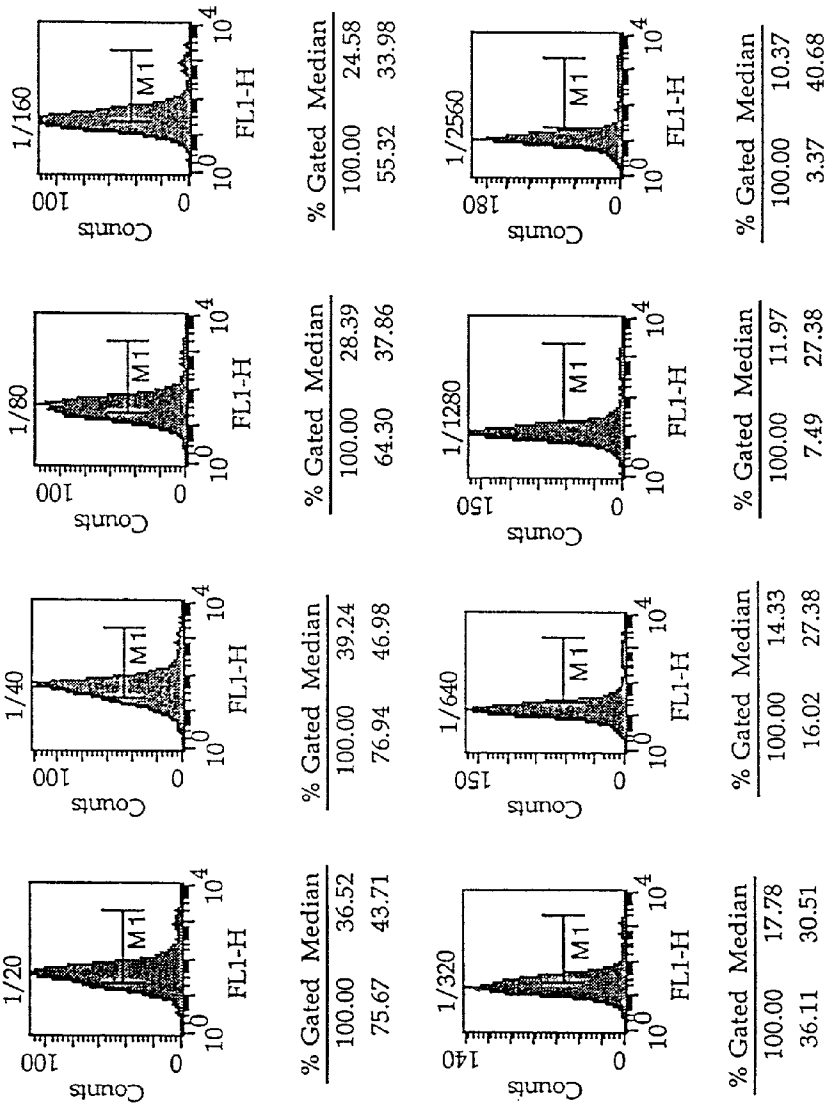


Fig.5.

TF CH/CD3A.44
(100µg/ml)



TF CH/CD3A.44 = humanised CD3A heavy chain with rat
CD3 VL + human Lambda light chain

ANTIBODY PREPARATION

[0001] The present invention relates to novel antibodies directed against the CD3 antigen complex, to DNA and RNA encoding for production these antibodies, to cell lines containing such DNA and/or RNA capable of producing them and to methods of producing the antibodies using the DNA, RNA and/or cells.

[0002] The human CD3 antigen consists of a minimum of four invariant polypeptide chains, which are non-covalently associated with the T-cell receptors on the surface of T-cells, and is generally now referred to as the CD3 antigen complex. This is intimately involved in the process of T-cell activation in response to antigen recognition by the T-cell receptors. All CD3 monoclonal antibodies can be used to sensitise T-cells to secondary proliferative stimuli such as IL1 (interleukin 1) and IL2 (interleukin 2). In addition, certain CD3 monoclonal antibodies are themselves mitogenic for T-cells. This property is isotype dependent and results from the interaction of the CD3 antibody Fc domain with Fc receptors on the surface of accessory cells.

[0003] Rodent CD3 antibodies have been used to influence immunological status by suppressing, enhancing or re-directing T-cell responses to antigens. They therefore have considerable therapeutic potential in the human for use as immuno-suppressive agents, for example for the treatment of rejection episodes following the transplantation of renal, hepatic and cardiac allografts.

[0004] WO 92/06193 and its equivalents (GB 2249310A, Appn No.s EP. 91917169.4, JP 516117/91 and U.S. Ser. No. 07/862,543; the contents of which are herein incorporated by reference) address the CD3 antibody antiglobulin response problem by re-shaping or "humanising" the variable region genes for the antibodies and expressing them in association with relevant human constant domain genes. This reduces the non-human content of the monoclonal antibody to such a low level that an antiglobulin response is unlikely.

[0005] WO 93/19196 and its equivalents (eg. EP 0586617, U.S. Pat. No. 5,585,097 and U.S. Ser. No. 08/478,684; the contents of which are herein incorporated by reference) address the problem of first dose response. These teach use of aglycosylated humanised CD3 antibodies of the IgG subclass which surprisingly retain their antigen binding specificity and immunosuppressive properties and yet do not induce T cell mitogenesis in vitro and induce a reduced level of cytokine release in vivo, whilst still maintaining some Fc binding ability.

[0006] Whilst these CD3 antibodies have great therapeutic value, their production in cell culture has not proven to be easy. In practice poor antibody yield is found accompanied by poor growth of transfected cell line. After much work over several years the best antibody levels achieved have been about 10 $\mu\text{g/ml}$, with cells expressing CD3 antibodies growing very slowly. Furthermore, these cells go negative over time in hollow cartridge systems used for large scale production.

[0007] The Celltech Glutamine Synthesis vector system PEE12 used in the aforesaid expression of CD3 antibodies routinely provides expression of other humanised antibodies at about 200 $\mu\text{g/ml}$. The original rat hybridoma cell line (YTH 12.5) expressed at a relatively normal level of 100

$\mu\text{g/ml}$ in cell culture, indicating poor antibody production to be associated with the humanised form. It appears that it is one or more of the expressed humanised proteins that proves toxic to the cells, as following transfection of cells they go negative faster than they grow.

[0008] The present inventors have now surprisingly found that by producing a chimeric form of the anti-CD3 antibody, linking the rat CD3 light chain variable region with the human lambda constant region and cloning this into PEE12 containing humanised CD3 aglycosyl heavy chain, they can produce myeloma cell lines which provide expression of functional aglycosylated CD3 antibodies at 60 to 100 $\mu\text{g/ml}$ of culture. By use of limiting dilution cloning some of the clones can be selected to provide still higher expression levels, eg. of the order of 120 $\mu\text{g/ml}$, and remain stable in long term culture with large scale production with no untoward effect on cell growth. Thus the present invention's chimeric antibodies offer good production capability without antiglobulin response normally associated with rat derived antibodies.

[0009] Accordingly, the present invention provides an IgG antibody having a binding affinity for the CD3 antigen complex in which in the heavy chain has a variable region framework together with at least one CDR selected from the amino acid sequences of SEQ ID No 2, 4 and 6 and respective conservatively modified variants thereof and the light chain has a variable region framework together with at least one CDR selected from the amino acid sequences of SEQ ID No 8, 10 and 12 and respective conservatively modified variants thereof

[0010] characterised in that the heavy chain variable region framework corresponds in sequence to the human type sequence and the light chain variable region includes one or more of the specific amino acids characteristic of the rodent type sequence.

[0011] Preferably the light chain variable region includes sufficient amino acids specific to the rodent type sequence such that the light and heavy chains associate more strongly than when the light chain variable region is of the corresponding fully human type. This can conveniently be such that the light chain variable region corresponds entirely to the rodent, eg. rat, sequence. Alternatively, only some or even one of the rat characteristic amino acids may be included.

[0012] Particular amino acids that are of rodent type rather than human type in the light chain variable region sequence are selected from those shown in SEQ ID No 14 in the sequence listing attached hereto, that being a preferred light chain variable region framework sequence wherein all the possible rat framework characteristic amino acids have been included, together with the respective CDR sequences. Thus amino acids characteristic of rat light chain variable framework region in SEQ ID No 14 are: Gln-1, Ala-2, Val-3, Val-4, Ala-7, Asn-8, Thr-12, Leu-14, Ser-16, Lys-19, Leu-20, Leu-39, Tyr-40, Glu-41, Ser-44, Met-48, Tyr-50, Phe-75, His-79, Asn-80, Val-81, Ala-82, Ile-83, Ile-88 and Phe-90. The corresponding human amino acids are in each case Asp-1, Phe-2, Met-3, Leu-4, Pro-7, His-8, Glu-12, Pro-14, Lys-15, Ile-19, Ile-20, Gln-39, Arg-40, Pro-41, Ala-44, Val-48, Phe-50, Ser-75, Ser-79, Gly-80, Leu-81, Gln-82, Thr-83, Asp-88 and Tyr-90. The latter human sequence is illustrated in EP 0586617 B on page 6 and in the corresponding US patent application.

[0013] Conveniently the heavy chain variable region framework is of human type and the light chain variable region is of rodent type, that being with all the aforespecified amino acids being the rat type of SEQ ID No 14. However, one or more, but not all of these positions of SEQ ID No 14 may be of the human type as long as sufficient rodent, eg. rat, sequence is present to enable stable light-heavy chain interaction to be achieved over that provided by the fully humanised form of the prior art. Such interaction is preferably such that when the antibody is expressed in PEE12 cells using suppliers (Celltech) instructions in excess of 50 µg/ml is achieved, more preferably in excess of 100 µg/ml. Preferably such cells should not go negative in significant numbers after several weeks use.

[0014] It will be realised by those skilled in the art that techniques such as site directed mutagenesis using PCR will allow the necessary production of these various light variable chains such that all embodiments of the invention may be produced without undue burden and screened for expression levels from the PEE12 cells.

[0015] The CDR amino sequences of SEQ ID No 2, 4, 6, 8, 10 and 12 correspond to CDRs (a), (b), (c), (d), (e) and (f) of WO 93/19196 and the CDRs themselves may also be referred to as respective CDRs (a) to (f) below.

[0016] Preferably the heavy chain and/or light chain each have all three of their respective CDRs of SEQ ID No 2, 4 and 6 and SEQ. ID. No 8, 10 and 12.

[0017] Preferably the antibody is aglycosylated. The term aglycosylated is employed in its normal usage to indicate that the antibodies according to the invention are not glycosylated.

[0018] By the term human type with respect to the framework region is meant a framework that is similar enough to human framework that it is substantially non-immunogenic in human when present in an intact antibody. Preferably an antibody of the invention having a heavy chain with a human type framework has between 60 and 140%, more typically at least 80 to 100%, of the rodent antibody affinity for the CD3 antigen. Characteristics of humanised monoclonal antibodies and methods for producing these from rodent monoclonals are disclosed in U.S. Pat. No. 5,585,089, the content of which is incorporated herein by reference for such purpose. Comparison of the human type heavy chain variable region with that of its rat counterpart can be made by comparing SEQ ID No 16 (rat) with the corresponding region found at the N-terminal of SEQ ID No 20. SEQ ID No 15 is that of DNA encoding for SEQ ID No 16. Thus a human type framework region may have, for example, seven or more of the thirteen changes that distinguish the sequence of the N-terminal 119 amino acids of SEQ ID No 20 from that of SEQ ID No 16. More preferably all the amino acids of the human type are incorporated. These changes may be at, for example, any of positions 5, 18, 19, 42, 49, 75, 77, 78, 88, 93, 97, 98 and 114 of these sequences.

[0019] By the term rodent type with respect to the framework region is meant a framework that correspond in amino acid sequence to that of an antibody of a rodent, eg. a rat or a mouse. In the case of anti-CD3 antibodies convenient framework amino acids are those of a rat antibody.

[0020] Further discussion of CD3 antigens is to be found in the report of the First International Workshop and Con-

ference on Human Leukocyte Differentiation Antigens and description of various glycosylated antibodies directed against the CD3 antigen is also to be found in the reports of this series of Workshops and Conferences, particularly the Third and Fourth, published by Oxford University Press. Specific examples of such antibodies include those described by Van Ller et al., *Euro. J. Immunol.*, 1987, 17, 1599-1604, Alegre et al., *J. Immunol.*, 1991, 140, 1184, and by Smith et al., *ibid*, 1986, 16, 478, the last publication relating to the IgG1 antibody UCHT1 and variants thereof.

[0021] However, of particular interest as the basis for antibodies according to the present invention are the CDRs contained in the antibodies OKT3 and YTH 12.5.14.2. The antibody OKT3 is discussed in publications such as Chatenaud et al., *Transplantation*, 1991, 51, 334 and the New England Journal of Medicine paper, 1985, 313, 339, and also in patents EP 0 018 795 and U.S. Pat. No. 4,361,539. The antibody YTH 12.5.14.2 (hereinafter referred to as YTH 12.5) is discussed in publications such as Clark et al., *European J. Immunol.*, 1989, 19, 381-388 and reshaped YTH 12.5 antibodies are the subject of EP 0504350 and its equivalents U.S. Ser. No. 08/362,780 and U.S. Pat. No. 5,585,097, these applications describing in detail the CDRs present in this antibody. The contents of U.S. Ser. No. 08/362,780, U.S. Pat. No. 5,585,097 and U.S. Pat. No. 4,361,539 are incorporated herein by reference.

[0022] The term "conservatively modified variants" is one well known in the art and indicates variants containing changes which are substantially without effect on antibody-antigen affinity. This term is conveniently defined as found in U.S. Pat. No. 5,380,712 which is incorporated herein by reference for such purpose.

[0023] Of the CDRs it is the heavy chain CDRs (a), (b) and (c) are of most importance. It will be realised by those skilled in the art that the antibodies of the invention also comprise constant domains.

[0024] The CDRs (a), (b) and (c) are arranged in the heavy chain in the sequence in the order: human framework region 1/(a)/human framework region 2/(b)/human framework region 3/(c)/human framework region 4 in a leader to constant domain (N-terminal to C-terminal) direction and the CDRs (d), (e) and (f) are arranged in the light chain in the sequence: rodent framework region 1/(d)/rodent framework region 2/(e)/rodent framework region 3/(f)/rodent framework region 4 in a leader to constant domain direction. It is preferred, therefore, that where all three are present the heavy chain CDRs are arranged in the sequence (a), (b), (c) in a leader to constant domain direction and the light chain CDRs are arranged in the sequence (d), (e), (f) in a leader to constant domain direction. The rodent framework region is preferably rat.

[0025] It should be appreciated however, that antibodies according to the invention may contain quite different CDRs from those described hereinbefore and that, even when this is not the case, it may be possible to have heavy chains and particularly light chains containing only one or two of the CDRs (a), (b) and (c) and (d), (e) and (f), respectively. However, although the presence of all six CDRs defined above is therefore not necessarily required in an antibody according to the present invention, all six CDRs will most usually be present in the most preferred antibodies.

[0026] A particularly preferred antibody therefore has a human type heavy chain with the three CDRs (a), (b) and (c)

comprising the amino acid sequences SEQ ID No 2, 4 and 6 or respective conservatively modified variants thereof and a rat light chain with the three CDRs (d), (e) and (f) comprising the amino acid sequences SEQ ID No 8, 10 and 12 or respective conservatively modified variants thereof in which the heavy chain CDRs are arranged in the order (a), (b), (c) in the leader constant region direction and the light chain CDRs are arranged in the order (d), (e), (f) in the leader constant region direction.

[0027] A preferred form of the first aspect of the present invention provides an antibody, particularly aglycosylated, which has a binding affinity for the human CD3 antigen in which the antibody constant region is of or is derived from one of human origin, particularly being the lambda constant region attached to the rat light chain variable region.

[0028] One convenient possibility is for the antibody to have a rat light chain variable domain framework region corresponding in amino acid sequence to that in the YTH12.5 hybridoma, ie. that of SEQ ID No 14, although the constant region will still preferably be of or derived from one of those of human origin, eg. will be the human lambda constant region. A preferred rat human chimeric light chain and lambda constant region amino acid sequence is that of SEQ ID No 18. Recombinant nucleic acid, eg. DNA, encoding for YTH12.5 comprises an amino acid sequence of SEQ ID No 13 while that encoding the rat light chain variable region and the human lambda constant region comprises SEQ ID No 17.

[0029] Certain human heavy chain variable domain framework sequences will be preferable for the grafting of the preferred CDR sequences, since the 3-dimensional conformation of the CDRs will be better maintained in such sequences and the antibody will retain a high level of binding affinity for the antigen. The heavy chain variable (V) region frameworks are preferably those coded for by the human VH type III gene VH26.D.J. which is from the B cell hybridoma cell line 18/2 (Huminghat, Dersimonian et al., Journal of Immunology, 139, 2496-2501; WO 93/19196 and U.S. Ser. No. 08/478,684)).

[0030] In a preferred form of the first aspect of the present invention the one or more preferred CDRs of the heavy chain of the rat anti-CD3 antibody are therefore present in a human variable domain framework which has the following amino acid sequence reading in the leader to constant region direction, CDR indicating a CDR (a), (b) or (c) as defined hereinbefore, a conservatively modified variant thereof or an alternative CDR:

[0031] SEQ ID No 21/CDR/SEQ ID No 22/CDR/SEQ ID No 23/CDR/SEQ ID No 24

[0032] Similarly, the one or more preferred CDRs of the light chain of the rat CD3 antibody are present in a rodent variable domain framework which has the following amino acid sequence reading in the leader to constant region direction, CDR indicating a CDR (d), (e) and (f) as defined hereinbefore, a conservatively modified variant thereof or an alternative CDR:

[0033] SEQ ID No. 25/CDR/SEQ ID No. 26/CDR/SEQ ID No. 27/CDR/SEQ ID No. 28.

[0034] In an aglycosylated antibody containing all three preferred light chain CDRs the light chain variable region comprises SEQ ID No 14:

[0035] The heavy and light chain constant regions can be based on antibodies of different types as desired subject to the antibody being an IgG antibody, but although they may be of or derived from those of rodent, eg. rat or mouse, origin they are preferably of or are derived from those of human origin. As described above, for the light chain the constant region is preferably of the lambda type and for the heavy chain it is preferably of an IgG isotype, especially IgG1, modified to effect aglycosylation as appropriate.

[0036] In an aglycosylated antibody containing all three preferred heavy chain CDRs, the heavy chain variable region and human IgG1 CH1-hinge aglycosylCH2CH3 comprises SEQ ID No 20 and is encoded for by DNA of SEQ ID No 19.

[0037] All human constant regions of the IgG isotype are known to be glycosylated at the asparagine residue at position 297, which makes up part of the N-glycosylation motif Asparagine²⁹⁷-X²⁹⁸-Serine²⁹⁹ or Threonine²⁹⁹, where X is the residue of any amino acid except proline. The antibody of the invention may thus be aglycosylated by the replacement of Asparagine²⁹⁷ in such a constant region with another amino acid which cannot be glycosylated. Any other amino acid residue can potentially be used, but alanine is the most preferred. Alternatively, glycosylation at Asparagine²⁹⁷ can be prevented by altering one of the other residues of the motif, e.g. by replacing residue 298 by proline, or residue 299 by any amino acid other than serine or threonine. Techniques for performing this site directed mutagenesis are well known to those skilled in the art and may for example be performed using a site directed mutagenesis kit such, for example, as that commercially available from Amersham. The procedure is further exemplified hereinafter.

[0038] It is well recognised in the art that the replacement of one amino acid in a CDR with another amino acid having similar properties, for example the replacement of a glutamic acid residue with an aspartic acid residue, may not substantially alter the properties or structure of the peptide or protein in which the substitution or substitutions were made. Thus, the aglycosylated antibodies of the present invention include those antibodies containing the preferred CDRs but with a specified amino acid sequence in which such a substitution or substitutions have occurred without substantially altering the binding affinity and specificity of the CDRs. Alternatively, deletions may be made in the amino acid residue sequence of the CDRs or the sequences may be extended at one or both of the N- and C-termini whilst still retaining activity.

[0039] Preferred aglycosylated antibodies according to the present invention are such that the affinity constant for the antigen is 10^5 mole⁻¹ or more, for example up to 10^{12} mole⁻¹. Ligands of different affinities may be suitable for different uses so that,

[0040] for example, an affinity of 10^6 , 10^7 or 10^8 mole⁻¹ or more may be appropriate in some cases. However antibodies with an affinity in the range of 10^6 to 10^8 mole⁻¹ will often be suitable. Conveniently the antibodies also do not exhibit any substantial binding affinity for other antigens. Binding affinities of the antibody and antibody specificity may be tested by assay procedures such as those described in the Examples section of EP 0586617 and U.S. Ser. No. 08/478,684 and U.S. Pat. No.

5,585,097, incorporated herein by reference, (See Example 5—Effector Cell Retargeting Assay), or by techniques such as ELISA and other immunoassays.

[0041] Antibodies according to the invention are aglycosylated IgG CD3 antibodies having a “Y” shaped configuration which may have two identical light and two identical heavy chains and are thus bivalent with each antigen binding site having an affinity for the CD3 antigen. Alternatively, the invention is also applicable to antibodies in which only one of the arms of the antibody has a binding affinity for the CD3 antigen. Such antibodies may take various forms. Thus the other arm of the antibody may have a binding affinity for an antigen other than CD3 so that the antibody is a bispecific antibody, for example as described in U.S. Pat. No. 4,474, 893 (incorporated herein by reference) and European Patent Applications Nos. 87907123.1 and 87907124.9. Alternatively, the antibody may have only one arm which exhibits a binding affinity, such an antibody being termed “monovalent”.

[0042] Monovalent antibodies (or antibody fragments) may be prepared in a number of ways. Glennie and Stevenson (Nature, 295, 712-713, (1982)) describe a method of preparing monovalent antibodies by enzymic digestion. Stevenson et al. describe a second approach to monovalent antibody preparation in which enzymatically produced Fab' and Fc fragments are chemically cross-linked (Anticancer Drug Design, 3, 219-230 (1989)). In these methods the resulting monovalent antibodies have lost one of their Fab' arms. A third method of preparing monovalent antibodies is described in European Patent No. 131424. In this approach the “Y” shape of the antibody is maintained, but only one of the two Fab' domains will bind to the antigen. This is achieved by introducing into the hybridoma a gene coding for an irrelevant light chain which will combine with the heavy chain of the antibody to produce a mixture of products in which the monovalent antibody is the one of interest.

[0043] More preferably, however, the monovalent aglycosylated CD3 antibodies of the invention are prepared by the following method. This involves the introduction into a suitable expression system, for example a cell system as described hereinafter, together with genes coding for the heavy and light chains, of a gene coding for a truncated heavy chain in which the variable region domain and first constant region domain of the heavy chain are absent, the gene lacking the exon for each of these domains. This results in the production by the cell system of a mixture of (a) antibodies which are complete bivalent antibodies, (b) antibody fragments consisting only of two truncated heavy chains (i.e. an Fc fragment) and (c) fragments of antibody which are monovalent for the CD3 antigen, consisting of a truncated heavy chain and a light chain in association with the normal heavy chain. Such an antibody fragment (c) is monovalent since it has any only one Fab' arm. Production of a monovalent antibody in the form of such a fragment by this method is preferred for a number of reasons. Thus, the resulting antibody fragment is easy to purify from a mixture of antibodies produced by the cell system since, for example, it may be separable simply on the basis of its molecular weight. This is not possible in the method of European Patent No. 131424 where the monovalent antibody produced has similar characteristics to a bivalent antibody in its size and outward appearance.

[0044] Additionally, the production of a monovalent antibody fragment by the new method uses conditions which can more easily be controlled and is thus not as haphazard as an enzyme digestion/chemical coupling procedure which requires the separation of a complex reaction product, with the additional advantage that the cell line used will continue to produce monovalent antibody fragments, without the need for continuous synthesis procedures as required in the enzyme digestion/chemical coupling procedure.

[0045] It is believed that aglycosylated antibodies according to the invention do not occur in nature and these aglycosylated antibodies may in general be produced synthetically in a number of ways. Most conveniently, however, appropriate gene constructs for the constant and variable regions of the heavy and light chains which are present in the antibody are separately obtained and then inserted in a suitable expression system.

[0046] Genes encoding the variable domains of a ligand of the desired structure may be produced and conveniently attached to genes encoding the constant domains of an antibody which have undergone site directed mutagenesis. These constant genes may be obtained from hybridoma cDNA or from the chromosomal DNA and have undergone site directed mutagenesis to produce the aglycosylated constant regions. Genes encoding the variable regions may also be derived by gene synthesis techniques used in the identification of the CDRs contained herein. Suitable cloning vehicles for the DNA may be of various types.

[0047] It will be realised by those skilled in the art that such genes may provided by a variety of methods. For example, it is possible to (i) raise a series of hybridomas against the CD3 antigen in the known manner, (ii) prepare DNA from these hybridomas by the procedures set out in WO 92/06193 and WO 93/19196 and their corresponding US patents by extracting mRNA and converting this to cDNA using PCR, (iii) screen this cDNA with oligonucleotide probes corresponding in sequence to CDR complementary DNA sequences, (iv) sequencing any positively identified hybridoma and (v) re-shaping the rat sequence by humanizing techniques set out in the aforesaid patents. In order to enable production of several and preferably all six preferred CDRs, site directed mutagenesis may be employed to insert desired DNA at corresponding points in the framework encoding DNA.

[0048] Expression of these genes through culture of a cell system to produce a functional CD3 ligand is most conveniently effected by transforming a suitable prokaryotic or particularly eukaryotic cell system, particularly an immortalised mammalian cell line such as a myeloma cell line, for example the YB2/3.01/Ag20 (hereinafter referred to as YO) rat myeloma cell, NS0 myeloma cell, or Chinese hamster ovary cells (although the use of plant cells is also of interest), with expression vectors which include DNA coding for the various antibody regions, and then culturing the transformed cell system to produce the desired antibody. Such general techniques of use for the manufacture of ligands according to the present invention are well known in the art and are described in publications such as “Molecular Cloning” by Sambrook, Fritsch and Maniatis, Cold Spring Harbour Laboratory Press, 1989 (2nd edition). The techniques are further illustrated by the Examples contained in WO 93/19196 and U.S. Ser. No 08/478,684, incorporated herein by reference.

[0049] A second aspect of the present invention thus provides a process for the preparation of an aglycosylated IgG antibody according to the first aspect having a binding affinity for the CD3 antigen which comprises culturing cells capable of expressing the antibody in order to effect expression thereof. A third aspect of the invention also provides a cell line which expresses an aglycosylated antibody according to the invention per se.

[0050] Preferred among such cell lines are those which comprise DNA sequences encoding the preferred CDRs described hereinbefore. A group of nucleotide sequences coding for the CDRs (a) to (f) described hereinbefore is as indicated under (a) to (f) below, respectively, but it will be appreciated that the degeneracy of the genetic code permits variations to be made in these sequences whilst still encoding for the CDRs' amino acid sequences.

[0051] (a) SEQ ID No 1; (b) SEQ ID No. 3; (c) SEQ ID No. 5; (d) SEQ ID No. 7;

[0052] (e) SEQ ID No. 9; (f) SEQ ID No. 11

[0053] Such cell lines will particularly contain larger DNA sequences which comprise (1) DNA expressing human heavy chain variable framework regions carrying one or more of (a), (b) and (c), and (2) DNA expressing rodent, eg. rat light chain variable framework regions carrying one or more of (d), (e) and (f).

[0054] A specific example of such DNA is SEQ ID No 19 which codes for the CDRs (a), (b) and (c) arranged in the heavy chain framework coded for by the human VH type III gene VH26.D.Jlinked to the human IgG. CH1-hinge-aglycosyl-CH2CH3 as discussed hereinbefore and that sequence SEQ ID No 17 which codes for the CDRs (d), (e) and (f) arranged in the light chain framework coded for by the YTH 12.5 human lambda constant region chimeric protein.

[0055] The chimeric partially humanised aglycosylated antibodies in accordance with the present invention have therapeutic value, particularly in immunosuppression, particularly in the control of graft rejection, where it is especially desirable that immunosuppression is temporary rather than total, and thus that T-cells are not completely destroyed, but instead rendered non-functional by antibody blockade of the CD3 antigen—TCR complex. In addition, the aglycosylated CD3 antibodies may have potential in other areas such as in the treatment of cancer, specifically in the construction of bispecific antibodies (for effector cell retargeting) or antibody-toxin conjugates, where the efficacy of the therapeutic agent would be compromised by Fc-mediated killing of the effector cells or non-specific killing of Fc receptor bearing cells respectively.

[0056] In a fourth aspect, the present invention thus includes a method of treating patients with cancer, particularly a lymphoma, or for immunosuppression purposes, for instance in a case where graft rejection may occur, comprising administering a therapeutically effective amount of an aglycosylated antibody in accordance with the first aspect of the invention.

[0057] Aglycosylated antibodies in accordance with the first aspect of the invention may be formulated for administration to patients by administering the said antibody together with a physiologically acceptable diluent or carrier. The antibodies are preferably administered in an injectable

form together with such a diluent or carrier which is sterile and pyrogen free. By way of guidance it may be stated that a suitable dose of antibody is about 1-10 mg injected daily over a time period of for example 10 days, although due to the elimination of the first dose response it will be possible if desired to administer higher amounts of the antibody, for example even up to 100 mg daily, depending on the individual patient's needs. Veterinary use is on a similar g/kg dosage basis.

[0058] The invention will now be described by way of illustration only by reference to the following non-limiting Examples, Figures and Sequence listing. Further embodiments of the invention falling within the scope of the claims will occur to those skilled in the art in the light of these.

FIGURES

[0059] FIG. 1: shows plots of FACS assay of binding of fully humanised aglycosyl CD3 (of EP0586617, U.S. Ser. No. 08/478,684 and U.S. Pat. No. 5,585,097) and a chimeric antibody of the present invention in which a rat light variable framework region is employed. Light chain YTH12.5LAG1 alone does not show normal binding as this is not associated with a heavy chain.

[0060] FIG. 2: shows plots of FACS assay of binding of two chimeric transfectants produced using pOXD52neo vector and thus which express CD52 antigen on their surfaces. These illustrate use of pOXCD52neo vector as a way of monitoring whether transfectants are a clonal population. TF 12.5L/CD3A.27 has one peak when stained with CD52 showing all cells are producing CD3 antibody, whereas TF12.5L/CD3A.34 has two peaks showing a negative population of cells that do not produce CD3 antibody.

[0061] FIG. 3: shows plots of OD₄₉₂ against dilution in an ELISA comparing human IgG production as a measure of antibody yield for present chimeric and prior art humanised aglycosylCD3. CD3 supernatants tested three weeks after transfection show the cell containing DNA encoding for a chimeric antibody of the present invention producing about 120 µg/ml and that the fully humanised CD3 producing less than 10 µg/ml.

[0062] FIGS. 4 and 5: show binding plots of the present invention chimeric antibodies and fully humanised CD3 to Jurkat cells for antibody affinity. Starting from a known concentration of 100 µg/ml and then diluting to 1/20 and seven fold titrations to 1/2560. The staining patterns demonstrate that the affinities are the same.

SEQUENCE LISTING

- [0063]** SEQ ID No 1 is that of DNA encoding for CDR (a).
- [0064]** SEQ ID No 2 is the amino acid sequence of CDR (a).
- [0065]** SEQ ID No 3 is that of DNA encoding for CDR (b).
- [0066]** SEQ ID No 4 is the amino acid sequence of CDR (b).
- [0067]** SEQ ID No 5 is that of DNA encoding for CDR (c).

- [0068] SEQ ID No 6 is the amino acid sequence of CDR (c).
- [0069] SEQ ID No 7 is that of DNA encoding for CDR (d).
- [0070] SEQ ID No 8 is the amino acid sequence of CDR (d).
- [0071] SEQ ID No 9 is that of DNA encoding for CDR (e).
- [0072] SEQ ID No 10 is the amino acid sequence of CDR (e).
- [0073] SEQ ID No 11 is that of DNA encoding for CDR (f).
- [0074] SEQ ID No 12 is the amino acid sequence of CDR (f).
- [0075] SEQ ID No 13 is that of DNA encoding for the rat light chain variable region.
- [0076] SEQ ID No 14 is the amino acid sequence of the rat light chain variable region.
- [0077] SEQ ID No 15 is that of DNA encoding the rat heavy chain variable region including the respective CDRs.
- [0078] SEQ ID No 16 is the amino acid sequence of the rat heavy chain variable region including the respective CDRs.
- [0079] SEQ ID No 17 is that of DNA encoding the rat light chain variable region with respective CDRs and the human lambda constant region.
- [0080] SEQ ID No 18 is the amino acid sequence of the rat light chain variable region with respective CDRs and the human lambda constant region.
- [0081] SEQ ID No 19 is the DNA sequence encoding for the heavy chain variable region with CDRs and the human CH1-hinge-aglycosylCH₂CH₃.
- [0082] SEQ ID No 20 is the amino acid sequence of the heavy chain variable region with CDRs and the human CH1-hinge-aglycosylCH₂CH₃.
- [0083] SEQ IDs No 21 to 24 are the amino acid sequences of the human heavy chain variable domain framework without CDRs.
- [0084] SEQ IDs No 25 to 28 are the amino acid sequences of the rat light chain variable domain framework without CDRs.
- [0085] SEQ ID No 29 and 30 are of primers used to clone rat CD3 light chain variable region into PEE12.

General Methodology

General Methods of Producing CD3 Specific Monoclonal Antibodies with Humanised Heavy Chain

[0086] The cloning and re-shaping of the V-region gene of the rat antibody YTH 12.5 specific for the human CD3 antigen is performed as described in Routledge et al., 1991, Eur. J. Immunol., 21, 2717 and in UK Patent Application No. 9121126.8 and its equivalents. YTH 12.5 is a rat hybridoma

cell line secreting an IgG2b monoclonal antibody specific for the CD3 antigen complex, but the methodology is applicable to other cells secreting CD3 specific antibodies with the same CDRs (see the preceding description).

[0087] Briefly, the methodology is based on that of Orlandi et al., 1989, PNAS USA, 86, 3833, using the polymerase chain reaction (PCR). The V_H gene (heavy chain variable region gene) is cloned using oligonucleotide primers VH1FOR and VH1BACK (see aforesaid incorporated patents). The PCR products are ligated into the vector M13-VHPCR1 in which site directed mutagenesis is performed using 6 oligonucleotide primers. The V_L gene (light chain variable region gene) was cloned using primers designed based on the published V_L sequences. The gene is cloned into the vector M13-VKPCR, together with the human lambda light chain constant region. In this vector mutagenesis of the V_L framework is performed using 5 oligonucleotides. The humanised V_L gene is then inserted into the expression vector pHApr-1.

[0088] Vector p316 is generated in which the reshaped CD3 VH gene may be expressed in conjunction with different immunoglobulin H chain constant region genes, this vector being based on the pHApr-gpt vector (Gunning et al., 1987, P.N.A.S. USA, 85, 7719-7723). A 1.65 Kb fragment of DNA carrying the dihydrofolate reductase (dhfr) gene and SV 40 expression signals (Page & Sydenham, 1991, Biotechnology, 9, 64) is inserted into the unique EcoRI site of pHApr-gpt. A 700 bp HindIII-BamHI DNA fragment encoding the reshaped CD3-VH gene is then cloned into the vector's multiple cloning site, downstream and under the control of the $\hat{a}$ actin promoter. The desired H chain constant region gene (in genomic configuration) can then be inserted into the unique BamHI restriction enzyme site downstream of the CD3-VH gene.

[0089] The aglycosyl human IgG1 constant region is derived from the wild type G1m (1,17) gene described by Takahashi et al., (1982, Cell, 29, 671-679) as follows. The gene is cloned into the vector M13 tg131 where site-directed mutagenesis is performed (Amersham International PLC kit) to mutate the amino acid residue at position 297 from an asparagine to an alanine residue.

[0090] Oligosaccharide at Asn-297 is a characteristic feature of all normal human IgG antibodies (Kabat et al., 1987, Sequence of Proteins of Immunological Interest, US Department of Health Human Services Publication), each of the two heavy chains in the IgG molecules having a single branched chain carbohydrate group which is linked to the amide group of the asparagine residue (Rademacher and Dwek, 1984, Prog. Immunol., 5, 95-112). Substitution of asparagine with alanine prevents the glycosylation of the antibody.

[0091] The 2.3 Kb aglycosyl IgG1 constant region is excised from M13 by double digestion using BamHI and BglIII and ligated into the BamHI site of vector p316 to produce clone p323.

[0092] Subconfluent monolayers of dhfr⁻ Chinese Hamster Ovary cells are co-transfected with the vector p323 containing the heavy chain gene and a second vector p274 containing the re-shaped human $\hat{e}$ light chain (Routledge et al., 1991, Eur. J. Immunol., 21, 2717-2725). Prior to transfection both plasmid DNAs were linearised using the restric-

tion endonuclease PvuI. Transfection is carried out using the DOTMA reagent (Boehringer, Germany) following the manufacturer's recommendations.

[0093] Heavy and light chain transfectants are selected for in xanthine/hypoxanthine free IMDM containing 5% (v/v) dialysed foetal calf serum.

[0094] The production of the analogous wild type human IgG1-CD3 heavy chain vector p278 has been described elsewhere (Routledge et al., 1991, Eur. J. Immunol., 21, 2717-2725 and GB9121126.8 incorporated herein by reference). H-chain expression vectors carrying the non-mutant human IgG2 (Flanagan & Rabbitts, 1982, Nature 300, 709-713), IgG3 (Huck et al., 1986, Nuc. Acid. Res., 14, 1779-1789), IgG4 (Flanagan & Rabbitts, 1982, Nature 300, 709-713), Epsilon (Flanagan & Rabbitts, 1982, EMBO. Journal 1, 655-660) and Alpha-2 (Flanagan & Rabbitts, 1982, Nature 300, 709-713) constant region genes (vectors p317, p318, p320, p321 and p325, respectively) are derived from the vector p316. Introduction of these vectors, in conjunction with the light chain vector p274, into dhfr⁻ CHO cells as described earlier, produced cell lines secreting CD3 antibody of the $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, α and $\alpha 2$ isotype respectively. Cells expressing CD3 antibodies were subjected to two rounds of cloning in soft agar, and then expanded into roller bottle cultures. The immunoglobulin from approximately 4 liters of tissue culture supernatant from each cell line is concentrated by ammonium sulphate precipitation, dialysed extensively against PBS and then quantified as follows:

[0095] As the antibody is not pure, a competition assay designed to specifically quantitate the concentration of antibody with CD3 antigen binding capacity was used. Human T-cell blasts are incubated with FITC labelled UCHT-1, an antibody which binds to the same epitope of the CD3 antigen as the chimeric panel. The concentration of FITC reagent used is previously determined to be half saturating. Unlabelled YTH 12.5 (HPLC purified) was titrated from a known starting concentration and added to wells containing T-cells and UCHT-1 FITC. The unlabelled antibody serves as a competitor for the antigen binding site. This is detected as decrease in the mean fluorescence seen when the cells are studied using FACS analysis. Thus, titration of the chimeric antibodies from unknown starting concentrations yields a series of sigmoidal curves when mean fluorescence is plotted against antibody dilution. These can be directly compared with the standard YTH 12.5 curve, an equivalent antibody may be used.

EXAMPLE 1

[0096] Preparation of an aglycosylated antibody specific for the human CD3 antigen, containing CDRs corresponding in sequence to those from the YTH 12.5 rat antibody, in human heavy chain variable framework linked to IgG1 constant region and rat light chain variable framework linked to human lambda constant region.

[0097] A chain loss variant of YTH12.5LAG1 was selected for loss of CD3 light chain, that only expressing rat CD3 heavy chain, and was used for the purpose of transfecting in fully humanised aglycosyl CD3 heavy chain. A 1.4 kb BamHI-HindIII DNA fragment encoding the humanised IgG1 aglycosyl CD3 heavy chain construct was cloned into the multiple cloning site of two different expression vectors, pH β Apr-1 gpt (Gunning et al (1987) P.N.A.S. USA 84, 4831 and 85, 7719-7723) and pOXCD52neo (Frewin unpublished) which contain different selectable markers.

[0098] pOXCD52neo expression vector is produced using the strong 'polypeptide chain elongation factor 1' promoter (EF1) which gives high-level antibody production (see Shigekazu Nagata NAR, Vol 18, No 17, page 5322. This is placed in a construct together with a neomycin selectable marker. Also included in the vector is a cDNA for Campath CD52 surface expressed antigen, driven by the TK promoter (all these promoters and markers are in the public domain by reason of availability). The expression of CD52 on the cell surface allows identification of transformants using CD52 antibodies.

[0099] YTH 12.5LAG1 was then transfected separately with the two plasmids by electroporation and heavy transfectants selected with IMDM containing 5% foetal calf serum, MPA and Xanthine for pH β Apr-Igpt and IMDM containing 5% foetal calf serum and 1 mg/ml G418 for pOXCD52neo, over a couple of weeks until live colonies grew up for testing. Both transfections yielded positive clones when screened for human IgG1 production using ELISA. Functional CD3 antibody was tested for by binding to a human T cell line Jurkat (ATCC TIB 152 (J. Immunol 133, 123-128 (1984)) and analysed by FACS (Becton Dickinson), both assays showing yields of antibody of between 30 and 50 μ g/ml.

[0100] The pOXCD52neo vector allows monitoring of transfected cells producing antibody with the use of a cell surface marker CD52. Only cells containing this marker secrete antibody so by taking Campath CD52 antibodies linked with FITC transfected cells can be analysed by FACS for the percentage of cells producing antibody and clonal status can be confirmed. No negative producing cells were detected and antibody yields remained at 50 μ g/ml with normal cell growth.

[0101] A chimeric form of the aglycosyl CD3 antibody was produced using PCR assembly to link the rat CD3 light chain variable region to the human lambda constant region using primers which introduce restriction enzyme sites Hind III and EcoRI to allow cloning into the Celltech expression vector PEE12 (see Bebbington et al (1992) Biotechnology 10, 169). The primer sequences are SEQ ID No 29 and 30 in the sequence listing attached hereto.

[0102] The final construct was sequenced and cloned into PEE12 already containing the humanised CD3 aglycosyl heavy chain and this was transfected into the myeloma cell line NS0 (ECACC No 85110503-Galfre and Milstein (1981) Enzymology 73 (B) 3-46) by electroporation. Resultant clones were screened for antibody production using ELISA for human IgG1 and human lambda light chain and on the FACS for binding to human T-cell clone Jurkat cell line. The ELISA uses goat anti-human IgFc (Sigma 12136) as capture antibody and Biotinylated sheep anti-human IgG (Amersham RPN 1003) or Biotinylated goat anti-human lambda light chain (Amersham RPN 1188) as detector antibody. (see Routledge et al Eur. J. Immunol (1991) 21: 2717-2725).

[0103] After one transfection 16 clones expressed 60 μ g/ml to 100 μ g/ml, far more than any other transfection with the reshaped aglycosyl CD3. These transfectants were then cloned by limiting dilution cloning and some of these improved to 120 μ g/ml. These remained stable in long term culture and large scale antibody production with no problems with cell growth.

[0104] FIGS. 1 to 4 illustrate the ability of these antibodies to bind CD3 with the same capacity as the previously described fully humanised aglycosyl anti-CD3 antibodies of the prior art.

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Gly	

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Gly

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Tyr Val His Trp Tyr Gln Leu Tyr Glu Gly Arg Ser Pro Thr Thr Met	
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85 90 95	
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 35 40 45
 Ile Tyr Asp Asp Asp Lys Arg Pro Asp Gly Val Pro Asp Arg Phe Ser
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 Gly Ser Ile Asp Arg Ser Ser Asn Ser Ala Phe Leu Thr Ile His Asn
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 Ser Met Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
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 cca atg gcc tgg gtc cgc cag gct cca aag aag ggt ctg gag tgg gtc 144
 Pro Met Ala Trp Val Arg Gln Ala Pro Lys Lys Gly Leu Glu Trp Val
 35 40 45
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 Ala Thr Ile Ser Thr Ser Gly Gly Arg Thr Tyr Tyr Arg Asp Ser Val
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 aag ggc cga ttc act atc tcc aga gat aat ggg aaa agc atc cta tac 240
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Ser Ile Leu Tyr
 65 70 75 80
 ctg caa atg aat agt ctg agg tct gag gac acg gcc act tat tac tgt 288
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Leu	Gln	Met	Asn	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Thr	Tyr	Tyr	Cys
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<212> TYPE: DNA															
<213> ORGANISM: Rattus															
<220> FEATURE:															
<221> NAME/KEY: CDS															
<222> LOCATION: (1)..(648)															
<400> SEQUENCE: 18															
cag	gct	ggt	gtg	act	cag	gca	aac	tct	gtg	tct	acg	tct	cta	gga	agc
Gln	Ala	Val	Val	Thr	Gln	Ala	Asn	Ser	Val	Ser	Thr	Ser	Leu	Gly	Ser
1				5					10				15		
aca	gtc	aag	ctg	tct	tgc	aca	ctc	agc	tct	ggg	aac	ata	gaa	aac	aac
Thr	Val	Lys	Leu	Ser	Cys	Thr	Leu	Ser	Ser	Gly	Asn	Ile	Glu	Asn	Asn
			20					25				30			
tat	gtg	cac	tgg	tac	cag	cta	tat	gag	gga	aga	tct	ccc	acc	act	atg
Tyr	Val	His	Trp	Tyr	Gln	Leu	Tyr	Glu	Gly	Arg	Ser	Pro	Thr	Thr	Met
			35				40					45			
att	tat	gat	gat	gat	aag	aga	ccg	gat	ggg	gtc	cct	gac	agg	ttc	tct
Ile	Tyr	Asp	Asp	Asp	Lys	Arg	Pro	Asp	Gly	Val	Pro	Asp	Arg	Phe	Ser
	50					55					60				
ggc	tcc	att	gac	agg	tct	tcc	aac	tca	gcc	ttc	ctg	aca	atc	cat	aat
Gly	Ser	Ile	Asp	Arg	Ser	Ser	Asn	Ser	Ala	Phe	Leu	Thr	Ile	His	Asn
65				70					75					80	
gtg	gca	att	gaa	gat	gaa	gct	atc	tac	ttc	tgt	cat	tct	tat	gtt	agt
Val	Ala	Ile	Glu	Asp	Glu	Ala	Ile	Tyr	Phe	Cys	His	Ser	Tyr	Val	Ser
				85					90					95	
agt	ttt	aat	gtt	ttc	ggc	ggg	gga	aca	aag	ctc	act	gtc	ctt	cga	cag
Ser	Phe	Asn	Val	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Thr	Val	Leu	Arg	Gln
			100					105					110		
ccc	aag	gct	gcc	ccc	tgg	gtc	act	ctg	ttc	ccg	ccc	tcc	tct	gag	gag
Pro	Lys	Ala	Ala	Pro	Ser	Val	Thr	Leu	Phe	Pro	Pro	Ser	Ser	Glu	Glu
			115				120					125			
ctt	caa	gcc	aac	aag	gcc	aca	ctg	gtg	tgt	ctc	ata	agt	gac	ttc	tac
Leu	Gln	Ala	Asn	Lys	Ala	Thr	Leu	Val	Cys	Leu	Ile	Ser	Asp	Phe	Tyr
	130					135					140				
ccg	gga	gcc	gtg	aca	gtg	gcc	tgg	aaa	gca	gat	agc	agc	ccc	gtc	aag
Pro	Gly	Ala	Val	Thr	Val	Ala	Trp	Lys	Ala	Asp	Ser	Ser	Pro	Val	Lys
145					150					155				160	
gcg	gga	gtg	gag	acc	acc	aca	ccc	tcc	aaa	caa	agc	aac	aac	aag	tac
Ala	Gly	Val	Glu	Thr	Thr	Thr	Pro	Ser	Lys	Gln	Ser	Asn	Asn	Lys	Tyr
				165					170					175	
gcg	gcc	agc	agc	tac	ctg	agc	ctg	acg	cct	gag	cag	tgg	aag	tcc	cac
Ala	Ala	Ser	Ser	Tyr	Leu	Ser	Leu	Thr	Pro	Glu	Gln	Trp	Lys	Ser	His
				180				185					190		

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aga agc tac agt tgc cag gtc acg cat gaa ggg agc acc gtg gag aag      624
Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
      195                200                205

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aca gtg gcc cct aca gaa tgt tca      648
Thr Val Ala Pro Thr Glu Cys Ser
      210                215

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<210> SEQ ID NO 19
<211> LENGTH: 216
<212> TYPE: PRT
<213> ORGANISM: Rattus

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<400> SEQUENCE: 19

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Gln Ala Val Val Thr Gln Ala Asn Ser Val Ser Thr Ser Leu Gly Ser
 1          5          10          15
Thr Val Lys Leu Ser Cys Thr Leu Ser Ser Gly Asn Ile Glu Asn Asn
      20          25          30
Tyr Val His Trp Tyr Gln Leu Tyr Glu Gly Arg Ser Pro Thr Thr Met
      35          40          45
Ile Tyr Asp Asp Asp Lys Arg Pro Asp Gly Val Pro Asp Arg Phe Ser
      50          55          60
Gly Ser Ile Asp Arg Ser Ser Asn Ser Ala Phe Leu Thr Ile His Asn
      65          70          75          80
Val Ala Ile Glu Asp Glu Ala Ile Tyr Phe Cys His Ser Tyr Val Ser
      85          90          95
Ser Phe Asn Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Arg Gln
      100         105         110
Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu Glu
      115         120         125
Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe Tyr
      130         135         140
Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val Lys
      145         150         155         160
Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys Tyr
      165         170         175
Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser His
      180         185         190
Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu Lys
      195         200         205
Thr Val Ala Pro Thr Glu Cys Ser
      210         215

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<210> SEQ ID NO 20
<211> LENGTH: 1347
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(1347)

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<400> SEQUENCE: 20

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gag gtc caa ctg ctg gag tct ggg ggc ggt tta gtg cag cct gga ggg      48
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1          5          10          15

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tcc ctg aga ctc tcc tgt gca gcc tca gga ttc act ttc agt agc ttt      96
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
      20          25          30

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cca atg gcc tgg gtc cgc cag gct cca ggg aag ggt ctg gag tgg gtc Pro Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35 40 45	144
tca acc att agt act agt ggt ggt aga act tac tat cga gac tcc gtg Ser Thr Ile Ser Thr Ser Gly Gly Arg Thr Tyr Tyr Arg Asp Ser Val 50 55 60	192
aag ggc cga ttc act atc tcc aga gat aat agc aaa aat acc cta tac Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr 65 70 75 80	240
ctg caa atg aat agt ctg agg gct gag gac acg gcc gtc tat tac tgt Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95	288
gca aaa ttt cgg cag tac agt ggt ggc ttt gat tac tgg ggc caa ggg Ala Lys Phe Arg Gln Tyr Ser Gly Gly Phe Asp Tyr Trp Gly Gln Gly 100 105 110	336
acc ctg gtc acc gtc tcc tca gcc tcc acc aag ggc cca tcg gtc ttc Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe 115 120 125	384
ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu 130 135 140	432
ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp 145 150 155 160	480
aac tca ggc gcc ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu 165 170 175	528
cag tcc tca gga ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser 180 185 190	576
agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro 195 200 205	624
agc aac acc aag gtg gac aag aaa gtt gag ccc aaa tct tgt gac aaa Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys 210 215 220	672
act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro 225 230 235 240	720
tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser 245 250 255	768
cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp 260 265 270	816
cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn 275 280 285	864
gcc aag aca aag ccg ccg gag gag cag tac gcc agc acg tac ccg gtg Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg Val 290 295 300	912
gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu 305 310 315 320	960
tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys 325 330 335	1008

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acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc	1056
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr	
340 345 350	
ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc	1104
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr	
355 360 365	
tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag	1152
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu	
370 375 380	
agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg	1200
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu	
385 390 395 400	
gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag	1248
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys	
405 410 415	
agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag	1296
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu	
420 425 430	
gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt	1344
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly	
435 440 445	
aaa	1347
Lys	

<210> SEQ ID NO 21

<211> LENGTH: 449

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly	
1 5 10 15	
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe	
20 25 30	
Pro Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	
35 40 45	
Ser Thr Ile Ser Thr Ser Gly Gly Arg Thr Tyr Tyr Arg Asp Ser Val	
50 55 60	
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr	
65 70 75 80	
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys	
85 90 95	
Ala Lys Phe Arg Gln Tyr Ser Gly Gly Phe Asp Tyr Trp Gly Gln Gly	
100 105 110	
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe	
115 120 125	
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu	
130 135 140	
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp	
145 150 155 160	
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu	
165 170 175	
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser	
180 185 190	

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Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
210 215 220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg Val
290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
340 345 350

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
420 425 430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
435 440 445

Lys

<210> SEQ ID NO 22
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
20 25 30

<210> SEQ ID NO 23
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
1 5 10

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<210> SEQ ID NO 24
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln
1 5 10 15

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys
20 25 30

<210> SEQ ID NO 25
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
1 5 10

<210> SEQ ID NO 26
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Rattus

<400> SEQUENCE: 26

Gln Ala Val Val Thr Gln Ala Asn Ser Val Ser Thr Ser Leu Gly Ser
1 5 10 15

Thr Val Lys Leu Ser Cys
20

<210> SEQ ID NO 27
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Rattus

<400> SEQUENCE: 27

Trp Tyr Gln Leu Tyr Glu Gly Arg Ser Pro Thr Thr Met Ile Tyr
1 5 10 15

<210> SEQ ID NO 28
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Rattus

<400> SEQUENCE: 28

Gly Val Pro Asp Arg Phe Ser Gly Ser Ile Asp Arg Ser Ser Asn Ser
1 5 10 15

Ala Phe Leu Thr Ile His Asn Val Ala Ile Glu Asp Glu Ala Ile Tyr
20 25 30

Phe Cys

<210> SEQ ID NO 29
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Rattus

<400> SEQUENCE: 29

Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Arg
1 5 10

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<210> SEQ ID NO 30
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: other
      nucleic acid - description: synthetic
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(36)

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<400> SEQUENCE: 30

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gactacaagc ttacacagga cctcaccatg cgatgg

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36

1. An IgG antibody having a binding affinity for the CD3 antigen complex in which in the heavy chain has a variable region framework together with at least one CDR selected from the amino acid sequences of SEQ ID No 2, 4 and 6 and respective conservatively modified variants thereof and the light chain has a variable region framework together with at least one CDR selected from the amino acid sequences of SEQ ID No 8, 10 and 12 respective conservatively modified variants thereof

characterised in that the heavy chain variable region framework corresponds in sequence to the human type sequence and the light chain variable region framework includes one or more of the specific amino acids characteristic of the rodent type sequence.

2. An IgG antibody as claimed in claim 1 characterised in that the light chain variable region includes sufficient amino acids specific to the rodent type sequence such that the light and heavy chains associate more strongly than when the light chain variable region is of the corresponding fully human type.

3. An IgG antibody as claimed in claim 1 or claim 2 characterised in that the light chain variable region includes sufficient amino acids specific to the rodent type sequence such that when DNA encoding for the antibody is expressed in PEE12 cells, an antibody yield of in excess of 50 µg/ml is obtained.

4. An antibody as claimed in any one of claims 1 to 3 characterised in that the light chain variable region corresponds entirely to the rat sequence.

5. An antibody as claimed in any one of the preceding claims characterised in that the light chain variable region corresponds to that of SEQ ID No 14 or that sequence altered such that one or more, but not all, of the amino acids Gln-1, Ala-2, Val-3, Val-4, Ala-7, Asn-8, Thr-12, Leu-14, Ser-16, Lys-19, Leu-20, Leu-39, Tyr-40, Glu-41, Ser-44, Met-48, Tyr-50, Phe-75, His-79, Asn-80, Val-81, Ala-82, Ile-83, Ile-88 and Phe-90 are substituted by corresponding human amino acids selected from Asp-1, Phe-2, Met-3, Leu-4, Pro-7, His-8, Glu-12, Pro-14, Lys-15, Ile-19, Ile-20, Gln-39, Arg-40, Pro-41, Ala-44, Val-48, Phe-50, Ser-75, Ser-79, Gly-80, Leu-81, Gln-82, Thr-83, Asp-88 and Tyr-90.

6. An antibody as claimed in any one of the preceding claims characterised in that the heavy chain and/or light chain each have all three of their respective CDRs of SEQ ID No 2, 4 and 6 and SEQ. ID. No 8, 10 and 12.

7. An antibody as claimed in any one of the preceding claims characterised in that the antibody is aglycosylated.

8. An antibody as claimed in any one of the preceding claims characterised in that the CDRs are arranged in the heavy chain in the sequence in the order: human framework region 1/SEQ ID No 2/human framework region 2/SEQ ID No 4/human framework region 3/SEQ ID No 6/human framework region 4 in a leader to constant domain (n-terminal to C-terminal) direction and in the light chain in the sequence: rodent framework region 1/SEQ ID No 8/rodent framework region 2/SEQ ID No 10/rodent framework region 3/SEQ ID No 12/rodent framework region 4 in a leader to constant domain direction.

9. An antibody as claimed in any one of the preceding claims characterised in that the human framework regions comprise amino acid sequences SEQ ID No 21, 22, 23 and/or 24

10. An antibody as claimed in any one of the preceding claims characterised in that the one or more preferred CDRs of the heavy chain of the rat anti-CD3 antibody are present in a human variable domain framework which has the amino acid sequence reading in the leader to constant region direction comprising

SEQ ID No 21/CDR/SEQ ID No 22/CDR/SEQ ID No 23/CDR/SEQ ID No 24.

11. An antibody as claimed in any one of the preceding claims characterised in that the antibody constant region is of human type.

12. An antibody as claimed in claim 11 characterised in that the antibody constant region corresponds to that of human origin.

13. An antibody as claimed in any one of the preceding claims characterised in that the antibody constant region comprises the human type lambda constant region.

14. An antibody as claimed in any one of the preceding claims characterised in that the rodent light chain variable region is attached to human type lambda constant region.

15. An antibody as claimed in any one of the preceding claims characterised in that it comprises a rat light chain variable domain framework region of SEQ ID No 14.

16. An antibody as claimed in any one of the preceding claims characterised in that it comprises a rat human chimeric light chain and lambda constant region amino acid sequence SEQ ID No 18.

17. An aglycosylated antibody according to any of claims 1 to 16, in which the heavy chain constant region is of an IgG1 isotype.

18. An aglycosylated antibody according any one of the preceding claims in which asparagine residue at position 297 of the constant region heavy chain is replaced by an alternative amino acid residue.

19. An aglycosylated antibody according to claim 18, in which the asparagine residue is replaced by an alanine residue.

20. An aglycosylated antibody according to any of the preceding claims, in which only one of the arms thereof has an affinity for the CD3 antigen.

21. An aglycosylated antibody according to claim 20 which is monovalent.

22. An aglycosylated antibody according to claim 21, in which one half of the antibody consists of a complete heavy chain and light chain and the other half consists of a similar but truncated heavy chain lacking the binding site for the light chain.

23. An aglycosylated antibody according to any one of the preceding claims characterised in that it is in the form of a pharmaceutical composition comprising a physiologically acceptable diluent or carrier.

24. An aglycosylated antibody according to any of claims 1 to 23 for use in therapy.

25. Use of an aglycosylated antibody according to any of claims 1 to 24 for the manufacture of a medicament for use in immunosuppression or treating cancer.

26. Recombinant nucleic acid encoding for an antibody as claimed in any one of the preceding claims.

27. Recombinant nucleic acid as claimed in claim 26 characterised in that it comprises a nucleotide sequence of SEQ ID No 13.

28. Recombinant nucleic acid as claimed in claim 26 or **27** characterised in that it comprises a nucleotide sequence of SEQ ID No 17.

29. Recombinant nucleic acid as claimed in any one of claims 26 to 28 characterised in that it encodes for a peptide of amino acid sequence SEQ ID No 20.

30. Recombinant nucleic acid as claimed in any one of claims 26 to 29 characterised in that it encodes for an amino acid sequence reading in the leader to constant region direction

SEQ ID No. 25/CDR/SEQ ID No. 26/CDR/SEQ ID No. 27/CDR/SEQ ID No. 28.

31. Recombinant nucleic acid as claimed in any one of claims 22 to 26 characterised in that it encodes for an amino acid sequence SEQ ID No 14

32. Recombinant nucleic acid as claimed in any one of claims 22 to 27 characterised in that it is DNA.

33. Recombinant nucleic acid characterised in that it encodes for a protein comprising an amino acid sequence SEQ ID No 18

34. Recombinant DNA of SEQ ID No 17.

35. A protein expression system characterised in that it comprises a recombinant nucleic acid as claimed in any one of claims 26 to 34.

36. A system as claimed in claim 35 characterised in that it comprises a vector incorporating nucleic acid as claimed in any one of claims 26 to 34.

37. A system as claimed in claim 35 or **36** characterised in that it comprises separate constructs of recombinant nucleic acid encoding for heavy and light chains respectively.

38. A system as claimed in claim 37 wherein the constructs encode for chains with constant regions.

39. A prokaryotic or eucaryotic cell expressing an antibody as claimed in any one of claims 1 to 24.

40. A cell as claimed in claim 39 characterised in that it comprises nucleic acid as claimed in any one of claims 26 to 34.

41. A cell as claimed in claim 39 or **40** characterised in that it is an immortalised human cell.

42. A process for producing an antibody as claimed in any one of claims 1 to 24 characterised in that it comprises culturing a cell as claimed in any one of claims 39 to 41.

43. A method of treating a patient having cancer or requiring immunosuppression which comprises administering to said patient a therapeutically effective amount of a ligand or an antibody or fragment thereof according to any of claims 1 to 24.

* * * * *