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(21) International Application Number: PCT/GB91/01578 (22) International Filing Date: 16 September 1991 (16.09.91) (30) Priority data: 9020282.1 17 September 1990 (17.09.90) GB (71)(72) Applicants and Inventors: GORMAN, Scott, David [US/GB]; Flat 22, Abington House, Adrian Way, Long Road, Cambridge CB2 2SA (GB). CLARK, Michael, Ronald [GB/GB]; 108 York Street, Cambridge CB1 2PY (GB). COBBOLD, Stephen, Paul [GB/GB]; Lower Flat, 22 Guest Road, Cambridge CB1 2AL (GB). WALDMANN, Herman [GB/GB]; 11 Gurney Way, Cambridge CB4 2ED (GB).	(74) Agent: MARCHANT, James, Ian; Elkington and Fife, Prospect House, 8 Pembroke Road, Sevenoaks, Kent TN13 1XR (GB). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report.</i>	
(54) Title: FRAMEWORK MUTATED ANTIBODIES AND THEIR PREPARATION (57) Abstract An altered antibody chain is produced in which the CDR's of the variable domain of the chain are derived from a first mammalian species. The framework-encoding regions of DNA encoding the variable domain of the first species are mutated so that the mutated framework-encoding regions encode a framework derived from a second different mammalian species. The or each constant domain of the antibody chain, if present, are also derived from the second mammalian species. An antibody which is capable of binding to human CD4 antigen is also provided together with a pharmaceutical composition comprising the antibody.		

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FRAMEWORK MUTATED ANTIBODIES AND THEIR PREPARATION

The present invention relates to altered antibodies and their preparation. The invention is typically applicable to the production of humanised antibodies.

5 Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains.
10 Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant
15 domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

 The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and
20 each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases
25 forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site.

 The preparation of an altered antibody in which the CDRs
30 are derived from a different species than the framework of the antibody's variable domains is disclosed in EP-A-0239400. The CDRs may be derived from a rat or mouse monoclonal antibody. The framework of the variable domains, and the constant domains, of the altered antibody
35 may be derived from a human antibody. Such a humanised

antibody elicits a negligible immune response when administered to a human compared to the immune response mounted by a human against a rat or mouse antibody.

Humanised CAMPATH-1 antibody is disclosed in EP-A-0328404.

5 We have now devised a new way of preparing an altered antibody. In contrast to previous proposals, this involves altering the framework of a variable domain rather than the CDRs. This approach has the advantages that it does not require a pre-existing cDNA encoding, for example, a human
10 framework to which to reshape and that it is technically easier than prior methodologies.

Accordingly, the present invention provides a process for the preparation of an antibody chain in which the CDRs of the variable domain of the antibody chain are derived
15 from a first mammalian species and the framework of the variable domain and, if present, the or each constant domain of the antibody chain are derived from a second different mammalian species, which process comprises:

(i) mutating the framework-encoding regions of DNA
20 encoding a variable domain of an antibody chain of the said first species such that the mutated framework-encoding regions encode the said framework derived from the said second species; and

(ii) expressing the said antibody chain utilising the
25 mutated DNA from step (i).

A variable domain of either or both chains of an antibody can therefore be altered by:

(a) determining the nucleotide and predicted amino acid sequence of a variable domain of a selected antibody chain
30 of the said first species;

(b) determining the antibody framework to which the framework of the said variable domain is to be altered;

(c) mutating the framework-encoding regions of DNA encoding the said variable domain such that the mutated

framework-encoding regions encode the framework determined upon in step (b);

(d) linking the mutated DNA obtained in step (c) to DNA encoding a constant domain of the said second species and
5 cloning the DNA into an expression vector; and

(e) introducing the expression vector into a compatible host cell and culturing the host cell under such conditions that antibody chain is expressed.

The antibody chain may be co-expressed with a
10 complementary antibody chain. At least the framework of the variable domain and the or each constant domain of the complementary chain generally are derived from the said second species also. A light chain and a heavy chain may be co-expressed. Either or both chains may have been
15 prepared by the process of the invention. Preferably the CDRs of both chains are derived from the same selected antibody. An antibody comprising both expressed chains can be recovered.

The antibody preferably has the structure of a natural
20 antibody or a fragment thereof. The antibody may therefore comprise a complete antibody, a (Fab')₂ fragment, a Fab fragment, a light chain dimer or a heavy chain. The antibody may be an IgG such as an IgG1, IgG2, IgG3 or IgG4 IgM, IgA, IgE or IgD. Alternatively, the antibody may be a
25 chimaeric antibody of the type described in WO 86/01533.

A chimaeric antibody according to WO 86/01533 comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain or heavy chain variable domain. Typically,
30 the chimaeric antibody comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused at its C-terminus to the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme region, a region derived from
35 a protein having known binding specificity, from a protein

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