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GEGEN CD3 GERICHTETE ANTIKÖRPER

ANTICORPS A EFFICACITE ANTIGONISTE A L'ANTIGENE CD3

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Description

Ihis invention relates to antibodies, in particular to re-shaped antibodies directed against the CD3 antigen on the surface of human T-cells.

Antibodies, or immunoglobulins, comprise two heavy chains linked together by disulphide bonds and two light chains, each light chain being linked to a respective heavy chain by disulphide bonds in a "Y" shaped configuration. The two "arms" of the antibody are responsible for antigen binding, having regions where the polypeptide structure varies, and are termed Fab' fragments (fragment - antigen - binding) or $F(ab')_2$ which represents two Fab' arms linked together by disulphide bonds. The "tail" or central axis of the antibody contains a fixed or constant sequence of peptides and is termed the Fc fragment (fragment - crystalline). The production of monoclonal antibodies was first disclosed by Kohler and Miistein (Kohler & Milstein, Nature, 256, 495-497 (1975)). Such monoclonal antibodies have found widespread use as diagnostic agents and also in therapy.

Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end, the light chain variable domain being aligned with the variable domain of the heavy chain and the light chain constant domain being aligned with the first constant domain of the heavy chain (CH1). The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen. The light chain constant region and the CH1 region of the heavy chain account for 50% of each Fab' fragment.

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 each domain comprises four framework regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs) (Kabat et al, Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services (1987)). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases 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.

In recent years, molecular biology techniques have allowed the production of a wide range of heterologous polypeptides by transformation of host cells with DNA sequences coding for the desired polypeptide. Immunoglobulin polypeptides have been produced by recombinant DNA techniques, see for example EP-A-0 088 994 (Schering Corporation), EP-A-1 102 634 (Takeda Chemical Industries Ltd.) and EP-A-0 125 023 (Genentech Inc.). These techniques have also allowed the stable introduction of immunoglobulin genes into myeloma cells.

When murine or rat monoclonal antibodies or even part human chimeric antibodies (antibodies where the antigen binding portion of an immunoglobulin is attached to at least part of another protein by a peptide linkage) comprising a mouse or rat variable domain is injected into a human in therapy, the human body's immune system could recognise that variable domain as foreign and thus produce an immune response. Hence, upon repeated injections of the mouse or rat monoclonal or chimeric antibody into humans, the effectiveness would be lost or reduced by the reaction of the body's immune system against the foreign antibody.

EP-A-O 239 400 (Winter) describes a monoclonal antibody in which only the CDRs of the antibody will be foreign to the body in order to minimise side effects due to its antigenicity if used for human therapy. Although, for example, human, mouse and rat framework regions have characteristic sequences, there seem to be no characteristic features which distinguish human from mouse and rat CDRs. Thus, an antibody comprised of mouse or rat CDRs in a human framework may well be no more foreign to the body than a genuine human antibody.

It is not clear however that the method of "humanizing" antibodies described in the above application will be suitable for application as a general method to all antibodies. Antibodies have either kappa or lambda light chains and one of alpha, mu, gamma, epsilon or delta heavy chains, specific combinations of which may make the above method of humanising antibodies inapplicable.

Until now, all of the humanised antibodies have contained a light chain of the kappa type. However, it has now been found possible to humanise an antibody directed against the human T-cell CD3 antigen (the monoclonal antibody secreted by the rat hybridoma YTH12.5.14.2 hereinafter referred to as YTH12.5), even though the antibody has a lambda type light chain. The presence of the lambda light chain required a different approach from that used for the humanisation of the mouse monoclonal antibody as described in EP-A-O 239 400.

Accordingly the present invention comprises a ligand, antibody, or antibody fragment with a binding affinity for the CD3 antigen, having a human constant region, a human or rat variable framework region, and at least one complementarity-determining region (CDR) selected from the amino acid sequences:

(a) Ser-Phe-Pro-Met-Ala,

(b) Thr-Ile-Ser-Thr-Ser-Gly-Gly-Arg-Thr-Tyr-Tyr-Arg-Asp-Ser-Val-Lys-Gly,

(c) Phe-Arg-Gln-Tyr-Ser-Gly-Gly-Phe-Asp-Tyr,

(d) Thr-Leu-Ser-Ser-Gly-Asn-Ile-Glu-Asn-Asn-Tyr-Val-His,

(e) Asp-Asp-Asp-Lys-Arg-Pro-Asp,

(f) His-Ser-Tyr-Val-Ser-Ser-Phe-Asn-Val,

and conservatively modified variants thereof.

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.

The CDRs of the invention are situated within framework regions of the heavy chain (for (a), (b) and (c)) and light chain (for (d), (e) and (f)) variable domains. The variable framework regions may be of human origin or of rat origin. In the latter case the variable framework region may, for example, be derived from the YTH 12.5 cell line. Preferably, however, the variable framework and constant regions are both of human origin.

Ligands according to the invention may contain varying numbers of CDRs. Thus, for example, the entities known as molecular recognition units contain a single CDR, but of rather greater interest among ligands which do not contain both a heavy and light chain are the single domain ligands described in European Patent Application No. 0 368 684 which contain three CDRs.

In a preferred embodiment of the invention, therefore, the ligand has three CDRs corresponding to the amino acid sequences (a), (b) and (c) above or conservatively modified variants thereof and/or three CDRs corresponding to amino acid sequences (d), (e) and (f) or conservatively modified variants thereof, the heavy chain CDRs (a), (b) and (c) being of most importance.

The present invention is however of particular interest in relation to whole antibodies or fragments thereof containing both heavy and light chain variable regions. Thus the ligand of the invention preferably has the form of an antibody or fragment thereof with a binding affinity for the CD3 antigen having a heavy chain with at least one CDR selected from the amino acid sequences:

(a) Ser-Phe-Pro-Met-Ala,

(b) Thr-Ile-Ser-Thr-Ser-Gly-Gly-Arg-Thr-Tyr-Tyr-Arg-Asp-Ser-Val-Lys-Gly,

(c) Phe-Arg-Gln-Tyr-Ser-Gly-Gly-Phe-Asp-Tyr,

and conservatively modified variants thereof, and/or a light chain with at least one CDR selected from the amino acid sequences:

(d) Thr-Leu-Ser-Ser-Gly-Asn-Ile-Glu-Asn-Asn-Tyr-Val-His,

(e) Asp-Asp-Asp-Lys-Arg-Pro-Asp,

(f) His-Ser-Tyr-Val-Ser-Ser-Phe-Asn-Val,

and conservatively modified variants thereof.

Although as indicated hereinbefore, ligands according to the invention do not have to contain both one or more of the specified heavy chain CDRs and one or more of the specified light chain CDRs, the antibodies or fragments thereof will usually do so. The CDRs (a), (b) and (c) are arranged in the rat hybridoma YTH12.5 heavy chain in the sequence: framework region 1/(a)/framework region 2/(b)/framework region 3/(c)/framework region 4 in a leader-→constant region direction and the CDRs (d), (e) and (f) are arranged in the hybridoma light chain in the sequence: framework region 1/(d)/framework region 2/(e)/framework region 3/(f)/framework region 4 in a leader → constant region 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 → constant region direction and the light chain CDRs are arranged in the sequence (d), (e), (f) in a leader → constant region direction.

It should be appreciated that 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 or fragment thereof according to the present invention, all six CORs will most usually be present. A particularly preferred antibody or fragment thereof therefore has a heavy chain with three CDRs comprising the amino acid sequences (a), (b) and (c) or conservatively modified variants thereof and a light chain with three CDRs comprising the amino acid sequences (d), (e) and (f) or 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.

The invention may be applied to antibodies having a "Y" shaped configuration which have two identical light and two identical heavy chains and are thus bivalent with each antigen binding site having an affinity for the C03 antigen. Alternatively, Fab' or $F(ab')_2$ fragments retaining the CDRs may be prepared. The invention is also applicable to antibodies and, where appropriate, fragments thereof, 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. Patent No. 4,474,893 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".

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.

More preferably, however, the monovalent CD3 antibodies of the invention are prepared by a new 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. 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.

The CDRs of the invention correspond to those present in the rat CD3 antibody YTH 12.5.

For the humanisation of the rat CDRs, certain human variable domain framework sequences will be preferable for combination with the CDR sequences according to the invention, 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. Desirable characteristics in such variable domain frameworks are the presence of key amino acids which maintain the structure of the CDR loops in order to ensure the affinity and specificity of the antibody for the CD3 antigen, the lambda type being preferred for the light chain.

We have identified human variable region frameworks which are particularly suitable for use in conjunction with the CDRs of the present invention. The heavy chain variable (V) region frameworks are those coded for by the human VH type III gene VH26.D.J. which is from the B cell hybridoma cell line 18/2 (Genbank Code: Huminghat, Dersimonian et al., Journal of Immunology, 139, 2496-2501). The light chain variable region frameworks are those of the human $V_L\lambda$ type VI gene SUT (Swissprot code: LV6CSHum, Solomon et al. In Glenner et al (Eds), Amyloidosis, Plenum Press N.Y., 1986, p.449.

The one or more CDRs of th heay chain of the rat anti-CD3 antibody are therefore preferably present in a human variable domain framework which has the following amino acid sequence reading in the leader → constant region direction, CDR indicating a CDR (a), (b) or (c) as defined hereinbefore, a conservatively modified variant thereof or an alternative CDR:-

Glu-Val-Gln-Leu-Leu-Glu-Ser-Gly-Gly-Gly-Leu-Val-Gln-Pro-Gly-Gly-
Ser-Leu-Arg-Leu-Ser-Cys-Ala-Ala-Ser-Gly-Phe-Thr-Phe-Ser-/CDR/-
Trp-Val-Arg-Gln-Ala-Pro-Gly-Lys-Gly-Leu-Glu-Trp-Val-Ser-/CDR/-
Arg-Phe-Thr-Ile-Ser-Arg-Asp-Asn-Ser-Lys-Asn-Thr-Leu-Tyr-Leu-Gln-
Met-Asn-Ser-Leu-Arg-Ala-Glu-Asp-Thr-Ala-Val-Tyr-Tyr-Cys-Ala-Lys-
/CDR/-Trp-Gly-Gln-Gly-Thr-Leu-Val-Thr-Val-Ser-Ser.

In a preferred antibody containing all three CDRs, the heavy chain variable region comprises the following sequence:-

Glu-Val-Gln-Leu-Leu-Glu-Ser-Gly-Gly-Gly-Leu-Val-Gln-Pro-Gly-Gly-
Ser-Leu-Arg-Leu-Ser-Cys-Ala-Ala-Ser-Gly-Phe-Thr-Phe-Ser-Ser-Phe-
Pro-Met-Ala-Trp-Val-Arg-Gln-Ala-Pro-Gly-Lys-Gly-Leu-Glu-Trp-Val-
Ser-Thr-Ile-Ser-Thr-Ser-Gly-Gly-Arg-Thr-Tyr-Tyr-Arg-Asp-Ser-Val-
Lys-Gly-Arg-Phe-Thr-Ile-Ser-Arg-Asp-Asn-Ser-Lys-Asn-Thr-Leu-Tyr-
Leu-Gln-Met-Asn-Ser-Leu-Arg-Ala-Glu-Asp-Thr-Ala-Val-Tyr-Tyr-Cys-
Ala-Lys-Phe-Arg-Gln-Tyr-Ser-Gly-Gly-Phe-Asp-Tyr-Trp-Gly-Gln-Gly-
Thr-Leu-Val-Thr-Val-Ser-Ser.

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

Asp-Phe-Met-Leu-Thr-Gln-Pro-His-Ser-Val-Ser-Glu-Ser-Pro-Gly-Lys-
Thr-Val-Ile-Ile-Ser-Cys-/CDR/-Trp-Tyr-Gln-Gln-Arg-Pro-Gly-Arg-Ala-
Pro-Thr-Thr-Val-Ile-Phe-/CDR/-Gly-Val-Pro-Asp-Arg-Phe-Ser-Gly-Ser-
Ile-Asp-Arg-Ser-Ser-Asn-Ser-Ala-Ser-Leu-Thr-Ile-Ser-Gly-Leu-Gln-
Thr-Glu-Asp-Glu-Ala-Asp-Tyr-Tyr-Cys-/CDR/-Phe-Gly-Gly-Gly-Thr-Lys-
Leu-Thr-Val-Leu.

In a preferred antibody containing all three CDRs the light chain variable region comprises the following sequence:-

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