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[54] **METHODS AND TRANSFORMED MAMMALIAN LYMPHOCYTE CELLS FOR PRODUCING FUNCTIONAL ANTIGEN-BINDING PROTEIN INCLUDING CHIMERIC IMMUNOGLOBULIN**

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Related U.S. Application Data

[63] Continuation of Ser. No. 893,610, Jun. 3, 1992, abandoned, which is a continuation of Ser. No. 675,106, Mar. 25, 1991, abandoned, which is a continuation of Ser. No. 441,189, Nov. 22, 1989, abandoned, which is a continuation of Ser. No. 90,669, Aug. 28, 1987, abandoned, which is a continuation-in-part of Ser. No. 644,473, Aug. 27, 1984, abandoned.

[51] **Int. Cl.**[6] **C12N 15/00**; C12N 15/13; C07K 16/00

[52] **U.S. Cl.** **435/69.6**; 435/172.3; 435/326; 530/387.1; 530/387.3; 536/23.53

[58] **Field of Search** 435/69.6, 246.27, 435/320.1, 172.3, 326; 530/387.3, 387.1; 935/15; 536/23.53

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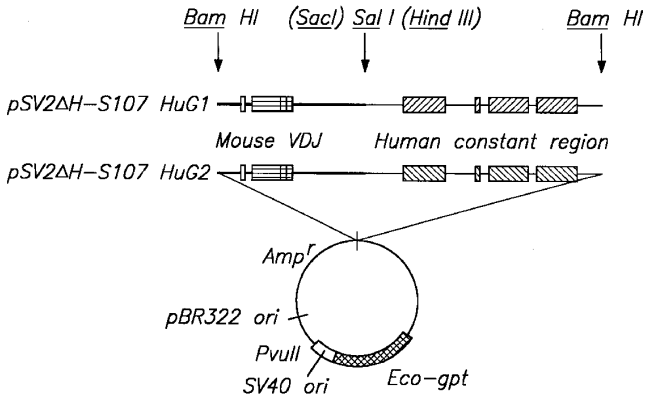
[57] **ABSTRACT**

Methods for producing functional immunoglobulin are provided. The methods involve transfecting and expressing exogenous DNA coding for the heavy and light chains of immunoglobulin. In some embodiments, chimeric immunoglobulins are provided having variable regions from one species and constant regions from another species by linking DNA sequences encoding for the variable regions of the light and heavy chains from one species to the constant regions of the light and heavy chains respectively from a different species. Introduction of the resulting genes into mammalian host cells under conditions for expression provides for production of chimeric immunoglobulins having the specificity of the variable region derived from a first species and the physiological functions of the constant region from a different species.

62 Claims, 2 Drawing Sheets



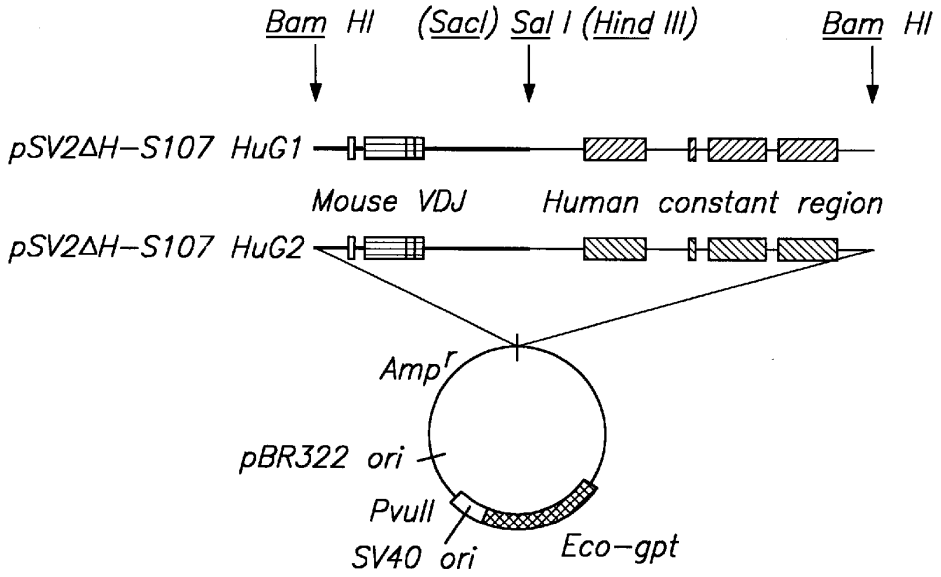


FIG. 1A

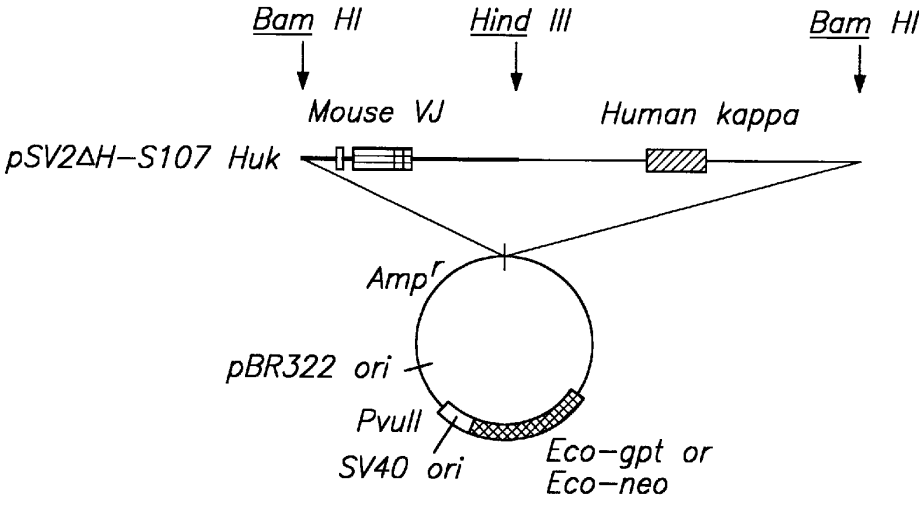


FIG. 1B

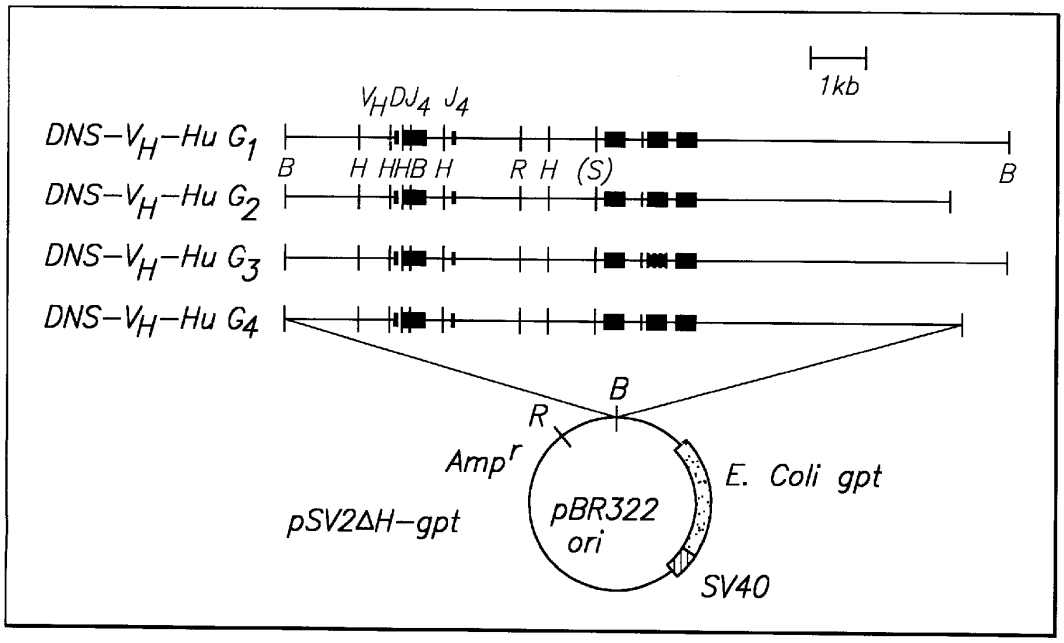


FIG. 2

METHODS AND TRANSFORMED MAMMALIAN LYMPHOCYTE CELLS FOR PRODUCING FUNCTIONAL ANTIGEN-BINDING PROTEIN INCLUDING CHIMERIC IMMUNOGLOBULIN

This is a continuation of application Ser. No. 07/893,610, filed Jun. 3, 1992, now abandoned, which is a continuation of application Ser. No. 07/675,106, filed Mar. 25, 1991, now abandoned, which is a continuation of application Ser. No. 07/441,189, filed Nov. 22, 1989, now abandoned, which is a continuation of application Ser. No. 07/090,669, filed Aug. 28, 1987, now abandoned, which is a continuation-in-part of application Ser. No. 06/644,473, filed Aug. 27, 1984 now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

Naturally occurring receptors, such as immunoglobulins, enzymes, and membrane proteins have seen an extraordinary expansion in commercial applications over the last decade. With the advent of monoclonal antibodies, the usefulness of immunoglobulins has been greatly expanded and in many situations has greatly extended prior uses employing polyclonal antibodies. However, in many applications, the use of monoclonal antibodies is severely restricted where the monoclonal antibodies are to be used in a physiological (in vivo) environment. Since, for the most part, monoclonal antibodies are produced in rodents, e.g., mice, the monoclonal antibodies are immunogenic to other species.

While the constant regions of immunoglobulins are not involved in ligand binding, the constant regions do have a number of specific functions, such as complement binding, immunogenicity, cell receptor binding, and the like. There will, therefore, be situations where it will be desirable to have constant regions which bind to cells or proteins from a particular species having binding regions for a particular ligand.

2. Relevant Literature

Kwan et al., *J. Exp. Mod.* (1981) 153:1366–1370 and Clarke et al., *Nucl. Acids Res.* (1982) 10:7731–7749 describe V_H and V_κ exons from the mouse phosphocholine-binding antibody-producing S107 myeloma cell line. Oi et al., *Proc. Natl. Acad. Sci. USA* (1983) 80:825–829, report that the mouse light chain gene is not expressed efficiently in a rat myeloma cell.

SUMMARY OF THE INVENTION

Chimeric multi-subunit receptors are provided, where each of the subunits is an expression product of a fused gene. Each fused gene comprises a DNA sequence from one host species encoding the region involved with ligand binding joined to a DNA sequence from a different source, either the same or a different host species, encoding a "constant" region providing a structural framework and biological properties. Introduction of the fused genes into an appropriate eukaryotic host cell under conditions for expression and processing provides for a functional assembled multi-subunit receptor product.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A is a schematic diagram of the chimeric mouse-:human heavy chain gene vector; and FIG. **1**B is the chimeric light chain vector.

FIG. **2** is a schematic diagram of chimeric human IgG anti-DNS expression vectors.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Novel methods and compositions are provided, for production of polypeptide products having specific binding affinities for a predetermined ligand and predetermined biological, particularly physiological, properties, each of which are not normally associated with the binding region peptide sequences. Particularly, multi-subunit chimeric receptors are provided which result from fused genes having the portion of the polypeptide involved with binding of a predetermined ligand having an amino acid sequence substantially the same (>90% conserved) as an amino acid sequence having the same function from one host, while the portion involved with providing structural stability, as well as other biological functions, being analogously derived from a different host. The resulting composition can be either an inter- or intraspecies chimera. At least two fused genes are involved, which genes are introduced into an appropriate eukaryotic host under conditions for expression and processing, whereby the fused genes are expressed and the resulting subunits bound together, resulting in an assembled chimeric receptor.

The receptors prepared in accordance with the subject invention will be multi-subunit, where the units are held together either by non-covalent binding or a combination of non-covalent and covalent binding, particularly disulfide linkages through cysteine, and having at least one binding site, usually at least two binding sites, and not more than about ten binding sites. Receptors of interest include both B-cell and T-cell receptors, more particularly, immunoglobulins, such as IgM, IgG, IgA, IgD and IgE, as well as the various subtypes of the individual groups. The light chain may be κ or λ. The heavy chains are referred to as μ, γ, α, δ, and ϵ.

In discussing the two regions of each subunit, the two regions will be referred to as "variable" and "constant" by analogy to immunoglobulins. The variable region is the region involved with ligand binding and, therefore, will vary in conformation and amino acid sequence depending upon the ligand. The region will usually be composed of a plurality of smaller regions (hypervariable or complementary determining regions), involving a region having as its primary function binding to the ligand (V) and a region associated with joining the V region to the constant region, the joining region (J). There may also be a hypervariable region joining the V and J regions, the diversity region (D). These regions are related to gene segments observed in the genes encoding immunoglobulin variable regions.

The constant region will not be associated with ligand binding and will be relatively limited in the variations in its conformation and amino acid sequence within any one species and within any one class, each class generally having from 1 to 4 subclasses. Each constant region is specific for a species. Within the classes there will be allotypes, individual polymorphisms within a class within a species.

The varible region of the immunoglobulins will be derived from a convenient mammalian source, which may be a rodent, e.g., mouse or rat, rabbit, or other vertebrate, mammalian or otherwise, capable of producing immunoglobulins. The constant region of the immunoglobulin, as well as the J chain for IgM and IgA (not the same as the J region of the heavy or light immunoglobulin chain), will be derived from a vertebrate source different from the source of the

variable region, particularly a mammalian source, more particularly primate or domestic animal, e.g., bovine, porcine, equine, canine, feline, or the like, and particularly, humans. The different source of the constant region can be either from a different species or from the same species as the mammalian source utilized to provide the variable region. Thus, the constant region of the receptor will normally be chosen in accordance with the purpose of the receptor. For example, where the receptor is to be introduced into the host, the constant portion will be selected so as to minimize the immune response of the host to the receptor and to optimize biological efficiency, such as complement fixation or physiological half-life (catabolism). Where the receptor is to bind to particular cell membrane surface receptors, the constant region will be chosen in accordance with the host of the receptor recognition site.

The fused gene derived from the two host sources will be prepared by joining the 5'-end of a sequence encoding the constant region in reading frame to the 3'-end of a sequence encoding the variable region. (In referring to 5' or 3' for a double strand, the direction of transcription is with 5' being upstream from 3'.) With immunoglobulins, two fused genes will be prepared, one for the light chain and one for the heavy chain. With T-cell receptors, the two fused genes will be for each of the two chains involved in the formation of the T-cell receptor. The DNA sequences employed for preparation of the fused gene may be derived from a variety of sources. These sources include genomic DNA, cDNA, synthetic DNA, and combinations thereof. The genomic DNA may or may not include naturally occurring introns.

The DNA obtained from natural sources, namely the genomic DNA or CDNA, may be obtained in a variety of ways. Host cells coding for the desired sequence may be isolated, the genomic DNA may be fragmented, conveniently by one or more restriction endonucleases, and the resulting fragments may be cloned and screened with a probe for the presence of the DNA sequence coding for the polypeptide sequence of interest. For the variable region, the rearranged germline heavy chain DNA will include V, D, and J regions, including the leader sequence, which may be subsequently removed as well as any introns. The rearranged germline light chain coding DNA will include the V and J regions including the leader sequence, as well as any introns which may be subsequently removed. The particular source of the exons defining the domains and the manner of splicing, where introns are present, is not germane to this invention. Once the cloned fragment has been identified which contains the desired DNA sequence, this fragment may be further manipulated to remove superfluous DNA, modify one or both termini, remove all or a portion of intervening sequences (introns), or the like.

In providing a fragment encoding the variable region, it will usually be desirable to include all or a portion of the intron downstream from the J region. Where the intron is retained, it will be necessary that there be functional splice acceptor and donor sequences at the intron termini. The gene sequence between the J (joining region) and the constant region of the fused gene may be primarily the intron sequence associated with (1) the constant region, (2) the J region, or (3) portions of each. The last may be a matter of convenience where there is a convenient restriction site in the introns from the two sources. In some instances, all or a portion of the intron may be modified by deletion, nucleotide substitution(s) or insertion, to enhance ease of manipulation, expression, or the like. When the variable region is chosen to be syngeneic with the host cells employed for expression, all or at least about 80% of the intron sequence can be selected from the naturally occurring intron sequence associated with the J region. In some instances it will be necessary to provide adapters to join the intron or truncated intron to the constant region. By cleaving within the intron, the variable region will be separated from its natural constant region.

Alternatively, it may be desirable to have the fused gene free of the intron between the variable and constant regions. Thus, the 3' terminus will be at or in the joining region. Normally all or a portion of the J region will be associated with the host providing the variable region. By restriction enzyme analysis or sequencing of the J region, one can select for a particular site for the 3' terminus of the variable region.

Alternatively, one can use an exonuclease and by employing varying periods of digestion, one can provide for varying 3'-termini, which can then be used for linking to the constant region and selection made for a functional product in a variety of ways. For example, where joining of the variable region to the constant region results in a unique restriction site, the fused DNA fragments may be screened for the presence of the restriction site.

Alternatively, it may be found desirable to include an adapter or linker to join the variable region to the constant region, where the adapter or linker may have the same or substantially the same sequence, usually at least substantially the same sequence, of the DNA sequence of the two fragments adjacent the juncture. The adapter or linker will be selected so as to provide for the two sequences to be in common reading frame. Furthermore, by employing adapters, one could add an additional degree of variability in the binding affinity of the chimeric receptor, by providing for the expression of different amino acids in the J region.

The joining of the various fragments is performed in accordance with conventional techniques, employing blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases.

For cDNA, the cDNA may be cloned and the resulting clone screened with an appropriate probe for cDNA coding for the desired variable or constant region. Once the desired clone has been isolated, the CDNA may be manipulated in substantially the same manner as the genomic DNA. However, with cDNA there will be no introns or intervening sequences. The cDNA is cleaved at or near the juncture of the variable region with the constant region so that the variable region is separated from the constant region and the desired region retained. Where a convenient restriction site exists, the CDNA may be digested to provide for a fragment having the appropriate terminus. The restriction site may provide a satisfactory site or be extended with an adapter. Alternatively, primer repair may be employed, where for the variable region a complementary sequence to the site of cleavage and successive nucleotides in the 3' direction of the complementary sequence is hybridized to the sense strand of the CDNA and the nonsense strand replicated beginning with the primer and removal of the single-stranded DNA of the sense strand 3' from the primer. The reverse is true for the constant region. Other techniques may also suggest themselves. Once the fragment has been obtained having the predetermined 3' or 5' terminus, as appropriate, it may then be employed for Joining to the other region.

Finally, one or both of the regions may be synthesized and cloned for use in preparing the fused gene. For the most part,

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