TABLE 1   CDR-GRAFTED GENE CONSTRUCTS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE	
			-	+
LIGHT CHAIN	ALL HUMAN FRAMEWORK RE1			
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+	n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d.	+
121B	26-32, 50-56, 91-96 inclusive + 46, 47	Partial gene assembly	n.d.	+
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+	+
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+	+
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+	+
221C	24-34, 50-56, 91-96 inclusive	Partial gene assembly	+	+
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL			
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d.	+
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d.	+
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+	n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+	n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly	+	
		Gene assembly		+
341	26-35, 50-65, 95-100B inclusive	SDM	+	
		Partial gene assembly		+
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63 = human) (SEQ ID NO:8-28)	Gene assembly	n.d.	+
341B	26-35, 50-65, 95-100B inclusive + 48, 49, 71, 73, 76, 78, 88, 91 (+63 + human)	Gene assembly	n.d.	+

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly

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14.   EXPRESSION OF CDR-GRAFTED GENES

14.1.   PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT
         (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMERIC
         HEAVY (cH) CHAINS

         All gL chains, in association with mH or cH
         produced reasonable amounts of antibody.
         Insertion of the Kozak consensus sequence at a
         position 5' to the ATG (kgL constructs) however,
         led to a 2-5 fold improvement in net expression.
         Over an extended series of experiments expression
         levels were raised from approximately 200ng/ml to
         approximately 500 ng/ml for kgL/cH or kgL/mH
         combinations.

         When direct binding to antigen on HUT 78 cells was
         measured, a construct designed to include mouse
         sequence based on loop length (gL121) did not lead
         to active antibody in association with mH or cH.
         A construct designed to include mouse sequence
         based on Kabat CDRs (gL221) (SEQ ID NO:26) demonstrated some weak
         binding in association with mH or cH.   However,
         when framework residues 1, 3, 46, 47 were changed
         from the human to the murine OKT3 equivalents
         based on the arguments outlined in Section 12.1
         antigen binding was demonstrated when both of the
         new constructs, which were termed 121A and 221A
         were co-expressed with cH.   When the effects of
         these residues were examined in more detail, it
         appears that residues 1 and 3 are not major
         contributing residues as the product of the gL221B
         gene (SEQ ID NO:27) shows little detectable binding activity in
         association with cH.   The light chain product of
         gL221C (SEQ ID NO:28), in which mouse sequences are present at 46
         and 47, shows good binding activity in association
         with cH.

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14.2   PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY
        (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMERIC
        LIGHT (cL) CHAINS
        Expression of the gH genes proved to be more
        difficult to achieve than for gL.   First,
        inclusion of the Kozak sequence appeared to have
        no marked effect on expression of gH genes.
        Expression appears to be slightly improved but not
        to the same degree as seen for the grafted light
        chain.
        Also, it proved difficult to demonstrate
        production of expected quantities of material when
        the loop choice (amino acid 26-32) for CDR1 is
        used, e.g. gH121, 131, 141 and no conclusions can
        be drawn about these constructs.   (SEQ ID NO:11)
        Moreover, co-expression of the gH341 gene with cL
        or mL has been variable and has tended to produce
        lower amounts of antibody than the cH/cL or mH/mL
        combinations.   The alterations to gH341 to (SEQ ID NO:11)
        produce gH341A and gH341B lead to improved levels
                  (SEQ ID NO:1)   (SEQ ID NO:2)
        of expression.
        This may be due either to a general increase in
        the fraction of mouse sequence in the variable
        region, or to the alteration at position 63 where
        the residue is returned to the human amino acid
        Valine (Val) from Phenylalanine (Phe) to avoid
        possible internal packing problems with the rest
        of the human framework.   This arrangement also
        occurs in gH331 and gH321.
        When gH321 or gH331 were expressed in association
        with cL, antibody was produced but antibody
        binding activity was not detected.   (SEQ ID NO:11)
        When the more conservative gH341 gene was used
        antigen binding could be detected in association
        with cL or mL, but the activity was only
        marginally above the background level.

When further mouse residues were substituted based
on the arguments in 12.1, antigen binding could be
clearly demonstrated for the antibody produced
when kgH341A and kgH341B were expressed in
association with cL.

14.3   PRODUCTION OF FULLY CDR-GRAFTED ANTIBODY
The kgL221A gene was co-expressed with kgH341,
kgH341A or kgH341B.   For the combination
kgH221A/kgH341 very little material was produced
in a normal COS cell expression.
For the combinations kgL221A/kgH341A or
kgH221A/kgH341B amounts of antibody similar to
gL/cH was produced.
In several experiments no antigen binding activity
could be detected with kgH221A/gH341 or
kgH221A/kgH341 combinations, although expression
levels were very low.
Antigen binding was detected when kgL221A/kgH341A
or kgH221A/kgH341B combinations were expressed.
In the case of the antibody produced from the
kgL221A/kgH341A combination the antigen binding
was very similar to that of the chimeric antibody.

An analysis of the above results is given below.

15.   DISCUSSION OF CDR-GRAFTING RESULTS
In the design of the fully humanised antibody the
aim was to transfer the minimum number of mouse
amino acids that would confer antigen binding onto
a human antibody framework.

15.1.   LIGHT CHAIN

15.1.1. EXTENT OF THE CDRs
For the light chain the regions defining the loops
known from structural studies of other antibodies
to contain the antigen contacting residues, and

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those hypervariable sequences defined by Kabat et
al (refs. 4 and 5) as Complementarity Determining
Regions (CDRs) are equivalent for CDR2.   For CDR1
the hypervariable region extends from residues
24-34 inclusive while the structural loop extends
from 26-32 inclusive.   In the case of OKT3 there *(SEQ ID NO:5)*
is only one amino acid difference between the two
options, at amino acid 24, where the mouse
sequence is a serine and the human framework RE1
has glutamine.   For CDR3 the loop extends from
residues 91-96 inclusive while the Kabat
hypervariability extends from residues 89-97
inclusive.   For OKT3 amino acids 89, 90 and 97 *29*
are the same between OKT3 and RE1 (Fig. 3). *(SEQ ID NO, 8 And 9)*   When
constructs based on the loop choice for CDR1
(gL121) and the Kabat choice (gL221) were made and
co-expressed with mH or cH no evidence for antigen
binding activity could be found for gL121, but
trace activity could be detected for the gL221,
suggesting that a single extra mouse residue in
the grafted variable region could have some
detectable effect.   Both gene constructs were
reasonably well expressed in the transient
expression system.

15.1.2. FRAMEWORK RESIDUES

The remaining framework residues were then further
examined, in particular amino acids known from
X-ray analysis of other antibodies to be close to
the CDRs and also those amino acids which in OKT3
showed differences from the consensus framework
for the mouse subgroup (subgroup VI) to which OKT3
shows most homology.   Four positions 1, 3, 46 and
47 were identified and their possible contribution
was examined by substituting the mouse amino acid
for the human amino acid at each position.
Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W,

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