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(54) **METHOD FOR REDUCING THE IMMUNOGENICITY OF ANTIBODY VARIABLE DOMAINS**

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(52) **U.S. Cl.** ..... **435/69.6**; 530/387.1; 530/387.3
(58) **Field of Search** ..... 530/387.1, 387.3; 435/69.6, 70.21; 424/130.1, 133.1

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(57) **ABSTRACT**

A unique method is disclosed for identifying and replacing immunoglobulin surface amino acid residues which converts the antigenicity of a first mammalian species to that of a second mammalian species. The method will simultaneously change immunogenicity and strictly preserve ligind binding properties. The judicious replacement of exterior amino acid residues has no effect on the ligind binding properties but greatly alters immunogenicity.

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| Position | Fractional Accessibility KOL Residue | Exposure | J539 Residue | Exposure | Residues In Subgroup I | II | III |
|---|---|---|---|---|---|---|---|
| 1 | E | 1.00 Ex | E | 1.00 Ex | Q | Q | E |
| 2 | V | 0.23 mB | V | 0.37 mB | V | V | V M |
| 3 | Q | 0.82 Ex | K | 0.82 Ex | Q | T Q | Q |
| 4 | L | 0.00 Bu | L | 0.10 Bu | L | L | L |
| 5 | V | 0.87 Ex | L | 1.00 Ex | V | R Q K T | V L |
| 6 | Q | 0.00 Bu | E | 0.09 Bu | Q | E | E |
| 7 | S | 0.94 Ex | S | 0.94 Ex | S | S | S |
| 8 | G | 1.00 Ex | G | 1.00 Ex | G | G | G |
| 9 | G | 0.00 Bu | G | 0.00 Bu | A | P | G |
| 10 | G | 1.00 Ex | G | 1.00 Ex | E | A G T | G A |
| 11 | V | 0.90 Ex | L | 0.81 Ex | V | L | L F |
| 12 | V | 0.25 mB | V | 0.25 mB | K | V | V |
| 13 | Q | 0.71 mE | Q | 0.87 Ex | K | K | Q |
| 14 | P | 0.59 PB | P | 0.64 mE | P | P | P |
| 15 | G | 1.00 Ex | G | 1.00 Ex | G | T S | G |
| 16 | R | 0.73 mE | G | 1.00 Ex | S A | E Q | G |
| 17 | S | 0.66 mE | S | 0.75 mE | S | T | S |
| 18 | L | 0.28 mB | L | 0.26mB | V | L | L |
| 19 | R | 0.66 mE | K | 0.75 mE | R K | T S | R K |
| 20 | L | 0.00 Bu | C | 0.00 Bu | V | L | L |
| 21 | S | 0.71 mE | S | 0.82 Ex | S | T | S |
| 22 | C | 0.00 Bu | C | 0.00 Bu | C | C | C |
| 23 | S | 1.00 Ex | A | 1.00 Ex | K | T | A |
| 24 | S | 0.00 Bu | A | 0.00 Bu | A T V | F V | A |
| 25 | S | 0.87 Ex | S | 1.00 Ex | S | S | S |
| 26 | G | 1.00 Ex | G | 1.00 Ex | G | G | G |
| 27 | F | 0.10 Bu | F | 0.10 Bu | G Y D | F L G | F |
| 28 | I | 0.85 Ex | D | 0.72 mE | T | S | T N |
| 29 | F | 0.00 Bu | F | 0.00 Bu | F | L I | F |
| 30 | S | 0.74 mE | S | 0.83 Ex | S N V I | S | S |
| 36 | W | 0.00 Bu | W | 0.00 Bu | W | W | W |
| 37 | V | 0.00 Bu | V | 0.00 Bu | V | I | V |
| 38 | R | 0.10 Bu | R | 0.31 mB | R | R | R |
| 39 | Q | 0.15 Bu | Q | 0.28 mB | Q | Q | Q |
| 40 | A | 0.95 Ex | A | 0.75 mE | A | P | A |
| 41 | P | 0.90 Ex | P | 0.73 mE | P | P | P S |
| 42 | G | 1.00 Ex | G | 1.00 Ex | G | G | G |
| 43 | K | 0.86 Ex | K | 0.86 Ex | Q R K H | K R | K |
| 44 | G | 1.00 Ex | G | 1.00 Ex | G | A G | G S |
| 45 | L | 0.00 Bu | L | 0.00 Bu | L | L | L |

FIG. 1a

| Position | Fractional Accessibility KOL Residue | Exposure | J539 Residue | Exposure | Residues In Subgroup I | II | III |
|---|---|---|---|---|---|---|---|
| 46 | E | 0.75 mE | E | 0.73 mE | E | E | E |
| 47 | W | 0.10 Bu | W | 0.04 Bu | W | W | W |
| 48 | V | 0.00 Bu | I | 0.00 Bu | MV | LI | V |
| 49 | A | 0.00 Bu | G | 0.00 Bu | G | AG | GSA |
| 66 | R | 0.36 mB | K | 0.51 pB | R | R | R |
| 67 | F | 0.00 Bu | F | 0.00 Bu | V | LV | F |
| 68 | T | 0.87 Ex | I | 0.88 Ex | T | T | T |
| 69 | I | 0.00 Bu | I | 0.00 Bu | VMI | IV | I |
| 70 | S | 0.78 mE | S | 0.79 mE | TS | ST | S |
| 71 | R | 0.11 Bu | R | 0.00 Bu | RLA | KV | R |
| 72 | N | 0.61 mE | D | 0.55 pB | DK | D | DN |
| 73 | D | 0.44 pB | N | 0.43 pB | PETAS | T | DN |
| 74 | S | 0.85 Ex | A | 0.97 Ex | S | S | S |
| 75 | K | 0.88 Ex | K | 0.77 mE | TF | KR | K |
| 76 | N | 0.69 mE | N | 0.68 mE | NST | N | N |
| 77 | T | 0.41 pB | S | 0.33 mB | TQ | Q | T |
| 78 | L | 0.00 Bu | L | 0.00 Bu | AV | VF | LA |
| 79 | F | 0.45 pB | Y | 0.35 mB | Y | VS | YF |
| 80 | L | 0.00 Bu | L | 0.00 Bu | M | L | L |
| 81 | Q | 0.53 pB | Q | 0.69 mE | E | TKSIN | Q |
| 82 | M | 0.00 Bu | M | 0.00 Bu | L | ML | M |
| 82a | D | 0.73 mE | S | 0.58 pB | SVRT | TSNIR | ND |
| 82b | S | 0.98 Ex | K | 0.96 Ex | S | NS | S |
| 82c | L | 0.00 Bu | V | 0.00 Bu | L | VM | L |
| 83 | R | 0.73 mE | R | 0.83 Ex | RFI | DT | RE |
| 84 | P | 0.75 mE | S | 0.90 Ex | S | PA | PA |
| 85 | E | 0.82 Ex | E | 0.90 Ex | E | VA | ED |
| 86 | D | 0.00 Bu | D | 0.11 Bu | D | D | D |
| 87 | T | 0.54 pB | T | 0.47 pB | T | T | T |
| 88 | G | 1.00 Ex | A | 0.00 Bu | A | A | A |
| 89 | V | 0.58 PB | L | 0.63 mE | V | TV | VL |
| 90 | Y | 0.00 Bu | Y | 0.00 Bu | Y | Y | Y |
| 91 | F | 0.00 Bu | Y | 0.08 Bu | Y | Y | Y |
| 92 | C | 0.00 Bu | C | 0.00 Bu | C | C | C |
| 93 | A | 0.00 Bu | A | 0.00 Bu | A | A | AT |
| 94 | R | 0.17 Bu | R | 0.15 Bu | R | RH | RP |

| | | | | | JH1 | JH2 | JH3 | JH4 | JH5 | JH6 |
|---|---|---|---|---|---|---|---|---|---|---|
| 103 | W | 0.09 Bu | W | 0.07 Bu | W | W | W | W | W | W |
| 104 | G | 0.00 Bu | G | 1.00 Ex | G | G | G | G | G | G |

FIG. 1b

| Position | Residue | Fractional Accessibility KOL Exposure | J539 Residue | J539 Exposure | Residues In Subgroup I JH1 | II JH2 | II JH3 | II JH4 | III JH5 | III JH6 |
|---|---|---|---|---|---|---|---|---|---|---|
| 105 | Q | 0.93 Ex | Q | 0.99 Ex | Q | R | Q | Q | Q | Q |
| 106 | G | 0.00 Bu | G | 0.00 Bu | G | G | G | G | G | G |
| 107 | T | 0.22 mB | T | 0.26 mB | T | T | T | T | T | T |
| 108 | P | 0.99 Ex | L | 0.67 mE | L | L | M | L | L | T |
| 109 | V | 0.00 Bu | V | 0.00 Bu | V | V | V | V | V | V |
| 110 | T | 0.76 mE | T | 0.69 mE | T | T | T | T | T | T |
| 111 | V | 0.00 Bu | V | 0.00 Bu | V | V | V | V | V | V |
| 112 | S | 0.98 Ex | S | 0.74 mE | S | S | S | S | S | S |
| 113 | S | 0.94 Ex | A | 0.84 Ex | S | S | S | S | S | S |

FIG. 1c

DOCKET ALARM

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