RESHAPING A HUMAN MONOCLONAL ANTIBODY TO INHIBIT HUMAN RESPIRATORY SYNCYTIAL VIRUS INFECTION *IN VIVO*

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We transferred the complementarity determining regions from a murine monoclonal antibody that neutralizes infection by respiratory syncytial virus (RSV) to a human IgG1 monoclonal antibody. The resulting reshaped human antibody lost affinity for RSV, but an additional alteration to one of the framework regions restored binding affinity and specificity. This second generation reshaped human monoclonal antibody cross-reacted with all clinical isolates of RSV tested and both prevented disease and cured mice even when administered four days after infection. We expect the antibody will prove useful in the management of this major childhood disease.

Respiratory syncytial virus (RSV) is the major cause of acute respiratory illness in young children admitted to hospitals, and the community practice will treat perhaps five times the number of hospitalized children. It therefore causes one of the major childhood diseases, giving rise to annual epidemics of bronchiolitis and pneumonia in children throughout the world[1,2]. While the majority of community-acquired infections resolve themselves in a week to ten days, many hospitalized children, especially under six months of age, require assisted ventilation. More severe disease may result in permanent damage to the lungs leading to pulmonary fibrosis. This can leave a child with increased susceptibility to chest infections such as chronic bronchitis, and indeed about 50% of the more seriously ill children go on to show recurrent bouts of wheezing in later years[3].

Efforts to produce an effective vaccine have been unsuccessful[4] and the major current treatment consists of intensive patient management involving the use of oxygen and possibly intragastric or intravenous feeding. Recently, the drug ribavirin has been introduced and has shown efficacy[5]. However, the drug has to be administered over an 18-hour period by aerosol inhalation. In addition, the level of secondary infections following cessation of treatment is significantly higher than in untreated patients.

Evidence that serum therapy may protect against RSV infection comes from a number of sources: (1) infants, less than one month old, show a low incidence of severe bronchiolitis and this has been interpreted as due to protecting maternal antibodies[6]; (2) human intravenous immunoglobulin (IVIG) prepared from high titer RSV-immune humans reduces nasal RSV shedding and improves oxygenation[7]; (3) a number of animal studies have shown that cotton rats and monkeys are protected against infection by passive administration of IVIG[8,9], and mouse monoclonal antibodies both protect against infection[10] and clear an established RSV infection in mice[11]. It is possible then that a single injection of high titer neutralizing monoclonal antibody will be more acceptable to the clinician to protect and possibly treat the child and prevent virus spread.

While mouse monoclonal antibodies with high neutralizing activity could be used in human therapy, application is severely limited by the immune response against mouse protein, reduced half-life of mouse antibodies in humans and poor recognition of mouse antibody effector domains by the human immune system[12,13]. Two genetic engineering techniques have been devised in an attempt to reduce immunogenicity. Chimaeric antibodies[14] were described in which the genetic information encoding the murine heavy and light chain variable regions are fixed to genes encoding the human heavy and light constant regions. The resulting mouse-human hybrid has about 30% of the intact immunoglobulin derived from murine sequences. In the second approach, only the genetic information for the hypervariable complementarity determining regions (CDRs) is derived from the murine antibody and transplanted in a human monoclonal antibody. With this technique, only about 3% of the amino acid sequence is of murine origin[15]. Here we have used this CDR grafting technique to redesign an anti-RSV mouse monoclonal

antibody as a human IgG1 monoclonal antibody and demonstrate the ability of this reshaped antibody to protect and cure mice from RSV infection.

RESULTS

V region sequence analysis of murine antibody. The original murine monoclonal antibody was RSV19 (IgG2a,K), specific for the fusion (F) protein of RSV. This antibody has been shown to neutralize RSV infection *in vitro* and protect mice (see below and E. J. Stott and G. T., in preparation). Cytoplasmic RNA was prepared from RSV19 hybridoma cells and cDNA from the immunoglobulin (Ig) mRNA using primers specific for Ig heavy chain variable (VH) regions and Ig light chain variable regions (VK) (see Experimental Protocol). VH and VK cDNAs were then amplified using the polymerase chain reaction and cloned into M13. Amplified DNAs from two separate cDNA preparations were sequenced in both directions from at least ten independent clones. All VH clones were identical as were VK clones except for one unrelated sequence, which contained a base pair deletion and consequently would be expected to be non-functional. The deduced amino acid sequences following DNA sequencing of clones is shown in Figure 1, with CDRs defined by computer assisted alignment with other VH and VK sequences[16]. The VH sequence is most closely related to Kabat subgroup IIC and the VK to Kabat subgroup II. The DNA sequence has been lodged with the EMBL data library.

Transplantation of CDR sequences into human frameworks. The human frameworks chosen to accept the CDR sequences were derived from NEWM for VH and REI for VK[17,18] and were within M13 based templates with irrelevant CDRs as described by Riechmann et al.[19] Synthetic oligonucleotides were synthesized containing the VH and VK CDRs flanked by short sequences drawn from NEWM and REI frameworks respectively and grafted into the human frameworks by site-directed mutagenesis[20]. The resulting constructs (HuRSV19VH/VK) are shown in Figure 2.

Expression of reshaped antibody. The reshaped VH and VK genes were transferred into vectors pSVgpt (conferring resistance to mycophenolic acid) and pSVhyg (conferring resistance to hygromycin) respectively[21] and human IgG1 and human kappa constant regions added. These were then cotransfected into YB2/0 rat myeloma cells and mycophenolic acid resistant clones selected and screened for antibody production. Antibody was purified by protein A affinity chromatography and analyzed by SDS-PAGE under reducing conditions. Two polypeptides of approximately 55kD and 25kD, corresponding to the heavy and kappa chains, were visible. Such clones secrete intact human IgG1 with yields of approximately 5 μg/ml/10^6 cells.

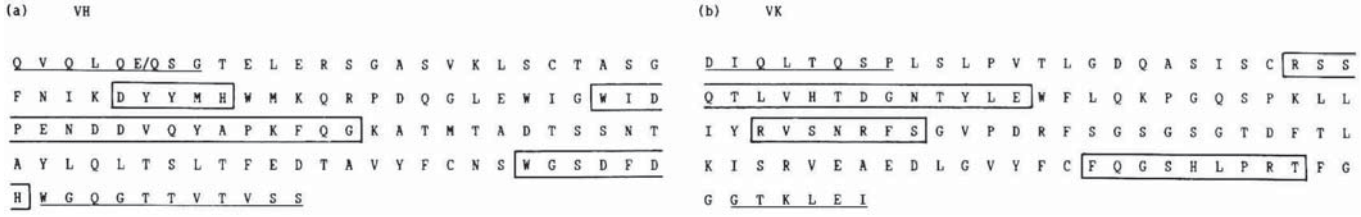
Antigen binding of reshaped antibodies. Figure 3 shows the binding of HuRSV19VH/VK antibody to antigen. Levels of binding were not significantly above background. Comparison of VH amino acid sequences between murine RSV19VH (Fig. 1) and HuRSV19VH (Fig. 2) shows that 3 out of 4 amino acid differences occur between amino acids 91–94. The majority of mouse and human VHs have arginine at position 94, which is able to form a salt bridge with aspartic acid at position 101 contributing to the conformation of CDR3[22]. Since the murine RSV19VH lacks Arg94 but has Asp101 we thought it possible that in HuRSV19VH an Arg94-Asp101 salt bridge has been imposed thereby altering the conformation and antigen interaction of CDR3. Accordingly, a second generation antibody was constructed, HuRSV19VHFNS/VK, with the murine amino acids 91–94 FCNS being used to replace the human VH framework amino acids YCAR (Fig. 2). It can be seen from Figure 3 that this resulted in the restoration of substantial antigen-binding ability. The restored binding affinity was lower than that with the original murine monoclonal antibody though direct comparison is difficult since different conjugated antibodies were used to detect mouse and human immunoglobulins.

TABLE 1 Binding of anti-RSV antibodies to clinical isolates.

Isolate Number	Extent of Fluorescence* HuRSV19VHFNS/VK	Murine RSV19
Subgroup A		
V818	++++	++++
V795	++++	++++
V00401	++	+++
V00214	+	++
V00764	++	+++
V743	++	+++
V316	++	++
V369	++++	++++
V1249	+++	+++
V04692	+++	+++
V1248	+	+
V01232	++	++
V729	+	++
Subgroup B		
V00634	+	++
V4715	++	+++
V00463	+	++
V4712	++	++
V00165	++	++
V00422	++	++
V837	+++	+++
V00900	++	++
4677	+++	+++
4424	++	++
V01231	+	+

*+, ++, +++ and ++++ refer to relative numbers of fluorescing cells observed and represent the proportion of cells infected.

FIGURE 1 Deduced amino acid sequences of (a) VH and (b) VK of murine RSV19 antibody. CDRs are boxed. Amino acids dictated by the PCR primers are underlined.



***In vitro* biological activity of reshaped antibody.** For clinical use, an antibody must recognize a high percentage of clinical isolates. Table 1 shows that the human antibody HuRSV19VHFNS/VK recognizes the same broad spectrum of isolates as the original murine antibody. Inhibition of virus induced cell fusion *in vitro* is a good indicator of *in vivo* protection[10]. Table 2 shows that the human antibody is effective at preventing the formation of giant cells associated with RSV-induced cell fusion. Moreover, residual giant cells in antibody treated cultures were smaller and had fewer nuclei per cell than in untreated cultures. The concentration of reshaped antibody which produced a 50% reduction in the number of multinucleated giant cells (6.3 μg/ml) was equivalent to that observed with the original murine antibody (4.0 μg/ml).

***In vivo* biological activity of reshaped antibody.** BALB/c mice were challenged intranasally with 10^4 pfu of A2 strain of human RSV[10] either before or after intranasal or intraperitoneal treatment with HuRSV19VHFNS/-VK human antibody. It can be seen from Table 3 that a single dose of 25 μg antibody per mouse is extremely effective in both prevention and treatment of RSV infection.

A comparison of the ability of the original mouse antibody and the reshaped human antibody to clear an established infection in BALB/c mice, when administered intraperitoneally on day 4 of infection, is shown in Figure 4. In this experiment, mice were infected with 10^5 pfu of virus, which is 10 times greater than that used in the experiment shown in Table 3. There were no significant differences in the ability of the two antibodies to remove virus from the lungs of mice. However, with the greater challenge dose of virus, approximately 5 mg/kg body weight were required to clear the infection.

DISCUSSION

The potential uses for human monoclonal antibodies in diagnosis, prophylaxis and therapy were immediately recognized with the first isolation in 1977 of a human cell line secreting specific antibody, but the development of an appropriate production technology has been a slow, laborious process[23–26]. Despite continuing research, a routinely applicable efficient methodology for isolating human monoclonals by mammalian cell transformation or cell fusion, techniques now so well established for rodent monoclonal production, is still not available. As an alternative, the use of genetic engineering has allowed the partial conversion of rodent antibodies into mouse-human chimaerics[14] and the use of protein engineering now allows the transfer of antigen binding specificity and affinity from a rodent antibody into a human immunoglobulin of any desired subtype[15,27–29].

The seminal studies on the use of CDR grafting to reshape human antibodies have already demonstrated the ability to transfer the properties required for successful depletion of T cells[19], inhibition of T-cell proliferation[30] and tumour imaging (M. Verhoeyen, personal communication). Our data has now shown that it is possible to produce a human monoclonal antibody with the composite of properties necessary for *in vivo* treatment of an infectious disease. The concentrations of human antibody

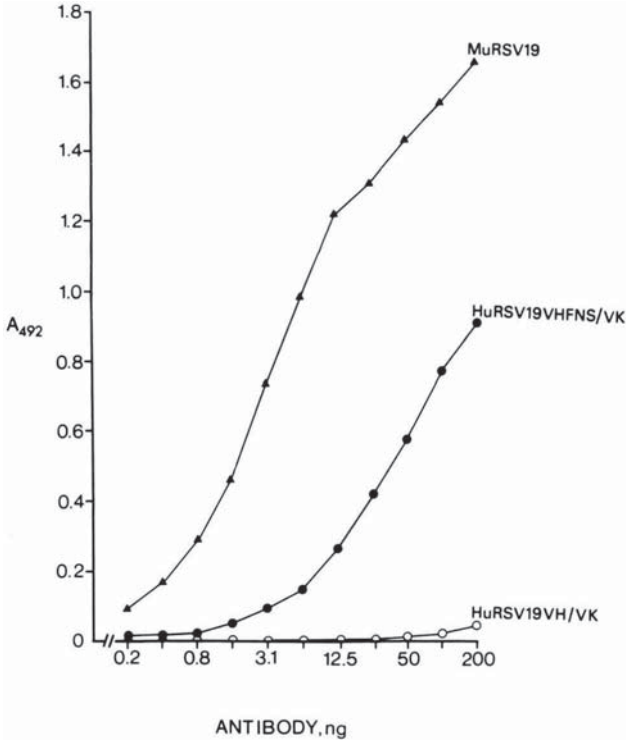


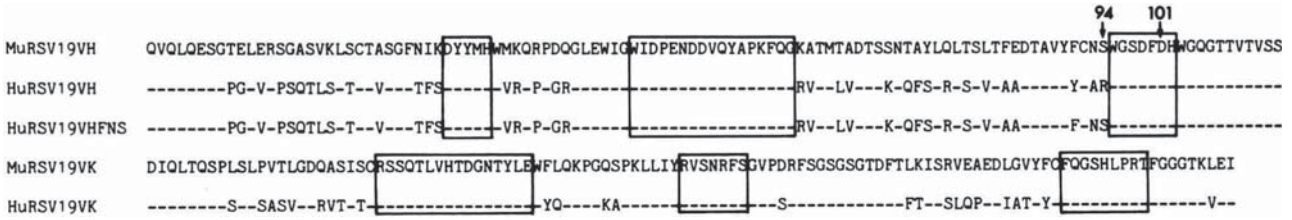
FIGURE 3 Antigen binding by murine RSV19, HuRSV19VH/VK and HuRSV19VHFNS/VK antibodies measured by ELISA.

TABLE 2 Inhibition of RSV induced cell fusion by reshaped anti-RSV antibody.

Concentration of HuRSV19VHFNS/VK (μg/ml)	Number of Giant Cells*	Average Number of Nuclei per Cell
100	44	4.5
50	71	4.0
25	40	3.8
12.5	67	N.D.
6.3	89	N.D.
3.1	87	N.D.
1.6	164	N.D.
0.8	201	N.D.
0.4	292	N.D.
0.2	219	N.D.
0 (virus only)	239,259	14,13.5
0 (no virus)	10	

*Scored as the number of cells with 2 or more nuclei in 20 fields with a 25× objective microscope lens.
N.D., not determined.

FIGURE 2 Amino acid sequences of murine and reshaped VH and VK containing RSV19 CDRs. Numbering is according to Kabat et al.[16]



required to inhibit *in vitro* virus-induced cell fusion and to treat mice *in vivo* (Tables 2 and 3) are equivalent to those needed with the original murine antibody (Fig. 4).

Structural and computer analysis of antigen antibody interactions have clearly demonstrated that individual framework residues can be critical for correct interactions of CDRs with antigen. For example, an arginine residue at position 94 in VH is thought to interact with the invariant aspartic acid residue at position 101 in the CDR[22]. It is not surprising then that simple transplantation of the CDRs as defined by Kabat from mouse to human often results in loss of binding affinity. This occurred with RSV19 (Fig. 3) and with several other antibodies undergoing reshaping

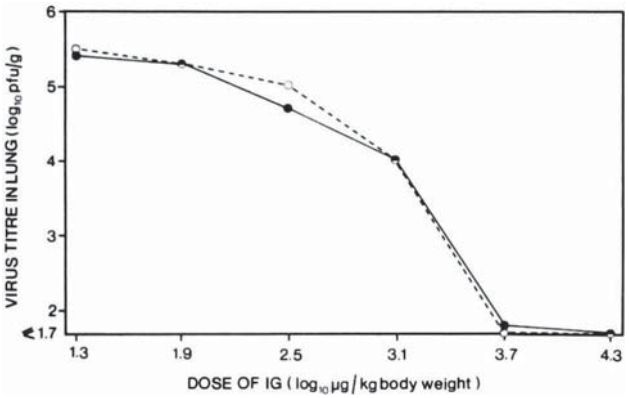


FIGURE 4 Effects of monoclonal antibodies on growth of RSV in lungs. HuRSV19VHFNS/VK (●) and murine RSV19 (○) given intraperitoneally 4 days after intranasal inoculation of mice with 10^5 pfu RSV strain A2.

TABLE 3 Prevention and treatment of RSV infection in mice by HuRSV19VHFNS/VK antibody.

Antibody Treatment		Virus Recovered (pfu)	Log pfu/g lung‡
Day*	Route		
−1	i.p.	0	<1.7
		0	<1.7
		0	<1.7
		0	<1.7
		0	<1.7
−1	i.n.	0	<1.7
		0	<1.7
		0	<1.7
		0	<1.7
		0	<1.7
+4	i.p.	0	<1.7
		0	<1.7
		0	<1.7
		0	<1.7
+4	i.n.	0	<1.7
		1	1.7
		0	<1.7
		1	1.7
		0	<1.7
No antibody	—	2950	4.47
		2100	4.32
		4400	4.64
		4150	4.61
		3600	4.55

*−1 refers to administration of HuRSV19VHFNS/VK antibody 1 day prior to RSV infection, +4 refers to administration of antibody 4 days post infection.
‡Virus pfu is expressed as the mean virus titre from 100 µl of either 10%, 1%, or 0.1% (w/v) lung homogenates adjusted to pfu per gram of lung.

in our laboratories. Ideally, reshaping of antibodies would be based upon structural data for each antigen-antibody interaction but this is clearly impractical. Our approach is to introduce ordered steps of additional alterations to achieve the minimal number of changes to the human structure necessary to restore binding affinity and specificity. Comparison of the amino acid sequences of the murine and human frameworks (Fig. 2) reveals a number of potential sites of framework-CDR interaction, and in this case, the interaction between CDR3 and its flanking framework residues was considered a significant structural variation (residues 91–94). An alternative to our approach[30] involves the construction of a consensus human structure based upon the best homology achievable with the original rodent antibody sequence. Which of these approaches is more effective at eliminating a human immune response remains to be clarified.

The projected advantages for human therapy of a reshaped human antibody compared with a rodent antibody are the absence of, or a considerably reduced, immune response allowing repeated treatment and an increased serum half-life, reducing the dose required and, in the case of prophylactics, extending the period of protection provided by a single treatment. The lack of an immune response has now been demonstrated with regard to one reshaped antibody constructed upon the same human framework used in our studies and antibody was still detectable *in vivo* eight days after administration[31]. These data also demonstrate the superiority in half-life and reduced immune response of the reshaped product compared to a chimaeric antibody[13].

The findings that our reshaped antibody is effective against a wide range of clinical isolates of RSV (Table 1) and both protects and cures mice (Table 3) raises hope that it will be clinically effective, thereby offering an additional weapon in the management of this major childhood infection.

EXPERIMENTAL PROTOCOL

Materials. Murine monoclonal antibody hybridoma cell line RSV19 was obtained from AFRC Institute for Animal Health, Compton, UK[10]. Rat myeloma YB2/0[32], obtained from ATTC, is a non-Ig secreting cell line and was grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum. Vectors M13VHPCR1, M13VKPCR1, pSVgpt and pSVhyg have been described in detail[21] and were obtained from G. Winter, MRC Laboratory of Molecular Biology, Cambridge, UK. Oligonucleotides were synthesized using an Applied Biosystems 381 DNA synthesizer. For cDNA synthesis, the primers were VH1FOR 5′ TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG 3′; VK1FOR 5′ GTTAGATCTCCAGCTTGHGTCCC 3′. For PCR, the additional primers were VH1BACK 5′ AGGTSMARCTGCAGSAGTCWGG 3′; VK1BACK 5′ GACATTCAGCTGACCCAGTCTCCA 3′. For site-directed mutagenesis to transplant CDRs, the primers used were: VHCDR1 5′ CTGTCTCACCCAGTGCATATAGTAGTCGCTGAAGGTGAAGCC AGACACGGT 3′, VHCDR2 5′ CATTGTCACTCTGCCCTG GAACTTCGGGGCATATGGAACATCATCATTCTCAGGA TCAATCCA 3′, VHCDR3 5′ CCCTTGGCCCCAGTGGTCAAAGTCACTCCCCCATCTTGCACAATA 3′, VKCDR1 5′ CTGCTGGTACCATTCTAAATAGGTGTTTCCATCAGTATGTACAAGGGTCTGACTAGATCTACAGGTGATGGTCA 3′, VKCDR2 5′ GCTTGGCACACCAGAAAATCGGTTGGAAACTCTGTAGATCAGCAG 3′, VKCDR3 5′ CCCTTGGCCGAACGTCCGAGGAAGATGTGAACCTTGAAAGCAGTAGTAGGT 3′. For site-directed mutagenesis of the human VH framework the oligonucleotide used was 5′ CTCCCCCATGAATTACAGAAATAGACCG 3′.

Murine variable region DNA sequencing. Cytoplasmic RNA was prepared as described by Favaloro et al.[33] The cDNA synthesis reaction consisted of 10–20 µg RNA, 0.4 µM VH1FOR or VK1FOR, 250 µM each of dATP, dCTP, dGTP and dTTP, 50 mM Tris-HCl, pH 7.5, 75 mM KCl, 10 mM DTT, 3 mM $MgCl_2$ and 27 units RNase inhibitor (Pharmacia) in a total volume of 50 µl. Samples were heated at 70°C for 10 min and slowly cooled to 42°C over a period of 30 min. Then, 100 units MMLV reverse

transcriptase (BRL) was added and incubation at 42°C continued for 1 hour. The VH and VK cDNAs were then amplified using the PCR as described by Orlandi et al.[21] For PCR amplification of VH, DNA/primer mixtures consisted of 5 μl RNA/cDNA hybrid, 0.5 μM VH1FOR and VH1BACK primers. For PCR amplifications of VK, DNA/primer mixtures consisted of 5 μl RNA/cDNA hybrid, 0.5 μM VH1FOR and VK1BACK primers. To these mixtures was added 200 μM each of dATP, dCTP, dGTP and dTTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 0.1% (v/v) Tween 20, 0.01% (v/v) NP40 and 2 units Taq DNA polymerase (United States Biochemicals) in a final volume of 50 μl. Samples were subjected to 25 thermal cycles of 94°C, 1 min; 60°C, 1 min; 72°C, 2 min; ending with 5 min at 72°C. Amplified VH and VK DNA were purified on low melting point agarose gels, and by Elutip-d column chromatography (Schleicher and Schuell) and cloned into M13. Clones were sequenced by the dideoxy method using Sequenase (United States Biochemicals).

Transplantation of CDRs into human frameworks. Oligonucleotide site-directed mutagenesis was based on the method of Nakamaya and Eckstein[20]. To 5 μg of VH or VK single-stranded DNA in M13 was added a two-fold molar excess of each of the three VH or VK phosphorylated oligonucleotides encoding the mouse CDR sequences. Primers were annealed to the template by heating to 70°C and slowly cooled to 37°C. After site-directed mutagenesis, the DNA was transformed into competent *E. coli* TG1 cells. Single-stranded DNA was prepared from individual plaques and sequenced. If only single or double mutants were obtained, then these were subjected to further rounds of mutagenesis using the appropriate oligonucleotides until the triple CDR mutants were obtained.

The CDR replaced VH and VK genes were cloned in expression vectors[21] to yield the plasmids termed pHuRSV19VH, pHuRSV19VHFNS and pHuRSV19VK. For pHuRSV19VH and pHuRSV19VHFNS, the CDR replaced VH gene together with the Ig heavy chain promoter, appropriate splice sites and signal peptide sequences was excised from M13 by digestion with HindIII and BamHI, and cloned into an expression vector containing the murine Ig heavy chain enhancer, the SV40 promoter, the *gpt* gene for selection in mammalian cells and genes for replication and selection in *E. coli*. A human IgG1 constant region[34] was then added as a BamHI fragment. The construction of the pHuRSV19VK plasmid was essentially the same except that the *gpt* gene was replaced by the hygromycin resistance gene and a human kappa chain constant region[35] was added.

Antibody expression. Ten μg pHuRSV19VH or pHuRSV19VHFNS and 20 μg pHuRSV19VK were linearized by digestion with PvuI. The DNAs were mixed together, ethanol precipitated and dissolved in 25 μl water. Approximately 10^7 YB2/0 cells were grown to semiconfluency, harvested by centrifugation and resuspended in 0.5 ml DMEM together with the digested DNA in an electroporation cuvette. After 5 min on ice, the cells were given a single pulse of 170V at 960 μF (Gene-Pulser, Bio-Rad) and left in ice for a further 20 min. The cells were then put into 20 ml DMEM plus 10% fetal calf serum and allowed to recover for 48 hours. At this time the cells were distributed into a 24-well plate and selective medium applied (DMEM, 10% fetal calf serum, 0.8 μg/ml mycophenolic acid, 250 μg/ml xanthine). After 3–4 days, the medium and dead cells were removed and replaced with fresh selective medium. Transfected clones were visible with the naked eye 8–10 days later.

The presence of human antibody in the medium of wells containing transfected clones was measured by ELISA. Microtiter plate wells were coated with goat anti-human IgG (gamma chain specific) antibodies (Sera-Lab). After washing with PBST (phosphate buffered saline containing 0.02% Tween 20, pH 7.5), 100 μl of culture medium from the wells containing transfectants was added to each microtiter well for 1 hour at 37°C. The wells were then emptied, washed with PBST and either peroxidase-conjugated goat anti-human IgG or peroxidase-conjugated goat anti-human kappa constant region antibodies (Sera-Lab) were added and incubated at 37°C for 1 hour. The wells were then emptied, washed with PBST and substrate buffer containing *o*-phenylenediamine added. Reactions were stopped after a few minutes by the addition of sulphuric acid and absorbance at 492 nm was measured.

Antigen binding assays. Humanized antibody secreted from transfected cell lines and the murine antibody secreted by the original hybridoma were purified by protein A affinity chromatography and tested for binding to RSV in an ELISA. Antigen consisted of calf kidney (CK) cells infected with the A2 strain of RSV and treated with 0.5% (v/v) NP40 detergent to yield a cell lysate. A control cell lysate was similarly prepared using uninfected CK cells. Microtiter plate wells were blocked with PBST and humanized or murine anti-RSV antibody applied. After 1 hour at 37°C wells were washed and biotinylated goat anti-human IgG or biotinylated goat anti-mouse IgG antibodies (Sera-Lab) added. After a further 1 hour incubation at 37°C peroxidase-conjugated streptavidin (Sera-Lab) was added for 20 min at 37°C. The wells were washed and peroxidase substrate buffer added. Reactions were stopped after a few minutes by the addition of sulphuric acid.

Immunofluorescence analysis of clinical isolates. Twenty-four clinical isolates were obtained from children during the winter of 1983/84 by the Bristol Public Health Laboratory, UK, and represented both of the major subgroups of RSV. Thirteen isolates were serotyped as subgroup A and 11 isolates as subgroup B. HeLa or MA104 cells infected with RSV isolates were grown in tissue culture. When the cells showed evidence of cytopathic effect, 20 ml 0.02% (w/v) disodium EDTA in PBS and 3 ml 0.25% (w/v) trypsin in PBS were added and the cell suspension spotted into wells of PTFE-coated slides. After 3 hours at 37°C, the slides were dried and fixed in 80% acetone. Cells were overlaid with anti-RSV antibody for 1 hour at room temperature. After extensive washing, either fluorescein-conjugated rabbit anti-mouse IgG (Nordic Laboratories) or fluorescein-conjugated goat anti-human IgG1 (Southern Biotechnology, Alabama) were added and incubation repeated. After further washing, cells were mounted in glycerol and examined under UV light.

***In vitro* analysis of inhibition of virus-induced cell fusion.** The reshaped antibody, HuRSV19VHFNS/VK was tested for biological activity *in vitro* in a fusion inhibition assay. A suspension of MA104 cells was infected with RSV at 0.1 pfu per cell. After 1 hour at 37°C, 2 ml of cells at 10^5/ml were distributed to glass coverslips in tubes. After a further 24 hours at 37°C, the culture medium was replaced by medium containing dilutions of reshaped antibody. Twenty-four hours later, coverslip cultures were fixed in methanol for 10 min and stained with May Grunwald stain (BDH).

***In vivo* analysis of efficacy.** BALB/c mice (MRC Clinical Research Centre, London: category 4, standard) were challenged intranasally with 10^4 pfu of the A2 strain of human RSV[37]. Groups of mice were administered 25 μg of reshaped antibody either one day prior to virus infection or 4 days following infection. Administration was either by the intranasal (i.n.) or intraperitoneal (i.p.) routes. Five days after RSV infection, mice were sacrificed and lungs assayed for RSV pfu, on secondary CK cells as described previously[10]. In a second experiment, mice were inoculated i.n. with 10^5 pfu of RSV, followed 4 days later with various concentrations of reshaped or murine antibody administered i.p. and examined on day 5 of infection for the level of RSV in the lungs.

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