#21

The studies in mice have been extended to humans. Human–human and human–mouse hybridomas have been prepared from patients with polyendocrine disease. These hybridomas synthesize autoantibodies that react with multiple normal human tissues (for example pituitary, gastric mucosa, pancreas and thyroid)[20]. Thus these antibodies may be a partial explanation for multiple organ autoimmunity in both animals and humans. A molecule in one organ may share common antigenic determinants with molecules in other organs. An antibody elicited against a common antigenic determinant in one organ would react with all organs containing that determinant, resulting in multiple organ autoimmunity. The availability of large quantites of monoclonal multiple organ-reactive autoantibodies should aid in isolating autoantigens and studying the molecular basis of autoimmunity.

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Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity

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The haemagglutinin (HA) glycoproteins of influenza virus membranes are responsible for binding viruses to cells by interacting with membrane receptor molecules which contain sialic acid (for review see ref. 1). This interaction is known to vary in detailed specificity for different influenza viruses (see, for example, refs 2–4) and we have attempted to identify the sialic acid binding site of the haemagglutinin by comparing the amino acid sequences of haemagglutinins with different binding specificities. We present here evidence that haemagglutinins which differ in recognizing either NeuAcα2→3Gal- or NeuAcα2→6Gal- linkages in glycoproteins also differ at amino acid 226 of HA1. This residue is located in a pocket on the distal tip of the molecule, an area previously proposed from considerations of the three-dimensional structure of the haemagglutinin to be involved in receptor binding[5].

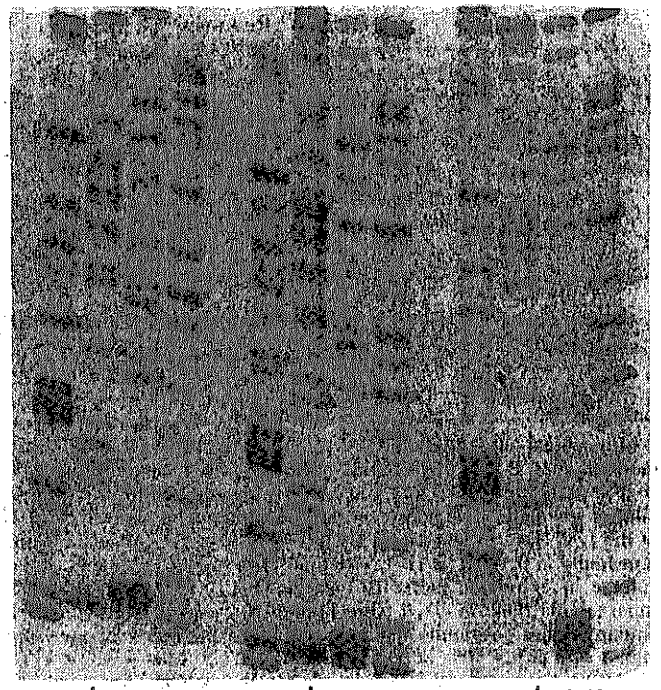

Fig. 1 Nucleotide sequence changes in the RNA of receptor binding variants. Sequences were determined using the dideoxynucleotide chain terminating procedure[8]. Each reaction mixture, 10 μl, contained Tris-Cl *p*H 8.3, 0.05 M; magnesium chloride, 0.012 M; dithiothreitol, 0.02 M; dATP, dCTP, dGTP, dTTP, 0.0004 M; human placenta RNase inhibitor (Bethesda Research Labs) 3 units; reverse transcriptase (Life Sciences) 5 units; and ddATP, ddCTP, ddGTP or ddTTP at either 0.00025 M or 0.00006 M and was incubated for 120 min at 42 °C. Products were analysed on polyacrylamide gels containing either 8% or 6% acrylamide. Reactions were primed using the ^{32}P-5′-labelled synthetic oligodeoxynucleotides[17]: 5AAAGCAGGGG14: 191TGCTACTGAGCT202: 345CGCAGCAAAG354: 493GCAAAAGGGG502: 623TCACCACCCG632: 777TGGACAATAG786: numbered according to the sequence of X-31 HA cDNA[10]. Those sequences shown from left to right are for viruses M1/HS8, M1/HS7 and M1/HS10 between nucleotides 738 and 780. The base changes are indicated by arrowheads and gel lanes contained from left to right ddGTP, ddATP, ddCTP and ddTTP terminated reactions. Nucleotide 742 which is a C is clearly separated from the two adjacent Cs only in the case of virus M1/HS10. Although this could indicate a C insertion in virus M1/HS10, this would alter the reading frame which would not be compatible with the fact that antigenically these viruses are indistinguishable and no other differences were detected between this variant and the wild type. For these reasons, coupled with the obvious differences in band intensity at this position in comparison with the other viruses, this region has been read as unchanged.

Comparison of receptor specificities of influenza viruses of the H3 subtype has revealed at least three distinct specificities based on preferential binding to either one or both of the sequences NeuAcα2→6Galα1-4GlcNAc- or NeuAcα2→3Galα1-3GalNAc- commonly found to terminate glycoprotein oligosaccharides linked to asparagine and to threonine or serine, respectively[6]. Preferential binding to the NeuAcα2→6Gal- linkage was also found to correlate with high sensitivity to neutralization of infection by glycoproteins (γ inhibitors) present in horse serum[7]. This fact has allowed selection of receptor-specific variants from the recombinant virus X-31 (H3N2).

Variants were selected by growth of X-31 virus in hen eggs in the presence of non-immune horse or guinea pig sera. Table 1 compares X-31 and the selected variants for inhibitor sensitivity and for receptor specificity using human erythrocytes

Table 1 Receptor specificity and horse serum inhibitor sensitivity of variants of influenza virus H3 haemagglutinins

Virus*	Haemagglutination of erythrocytes†				Haemagglutination‡ inhibition HAI	Amino acid at residue 226§
	Native	Sialidase treated	NeuAcα2→3Gal	NeuAcα2→6Gal		
	HA titre					
X-31	4,096	0	0	4,096	5,000	Leu
X-31/HS	4,096	0	4,096	0	30	Gln
X-31/GPS	512	0	256	0	30	Gln
M1/5	64	0	0	64	4,200	Leu
M1/HS10	64	0	0	64	8,100	Leu
M1/HS7	64	0	32	64	500	Met
M1/HS8	64	0	32	0	30	Gln

* Influenza virus X-31 (H3N2) was grown in hens' eggs in the absence (X-31) or presence of horse serum (X-31/HS) or guinea pig serum (X-31/GPS) and was purified as previously described[16]. Cloned variants of A/Memphis/102/72 (H3N2) were produced from a seed stock, obtained from Dr Robert G. Webster, by passage in MDCK cells in the absence (M1/5) or presence (M1/HS) of horse serum and subsequent plaque to plaque purification. HA was done either with MDCK cell lysates or with virus grown in hens' eggs. The titres are expressed as the reciprocal of the greatest dilution of virus at which agglutination occurred.

† Human erythrocytes were either unmodified (Native), treated with *Vibrio cholera* sialidase (sialidase treated) or sialidase treated and resialylated with CMP-NeuAc and purified sialytransferases to contain the NeuAcα2→3Galβ1→3GalNAc or NeuAcα2→6Galβ1→4GlcNAc sequences as reported earlier[4].

‡ Haemagglutinin inhibition (HAI) with horse serum was done by standard procedures with HAI titres expressed as the reciprocal of the greatest dilution that prevented agglutination by 4 HA units of virus.

§ Deduced from the nucleotide sequences of the HA1 region of the haemagglutinin genes. These were the only amino acid changes detected in HA1.

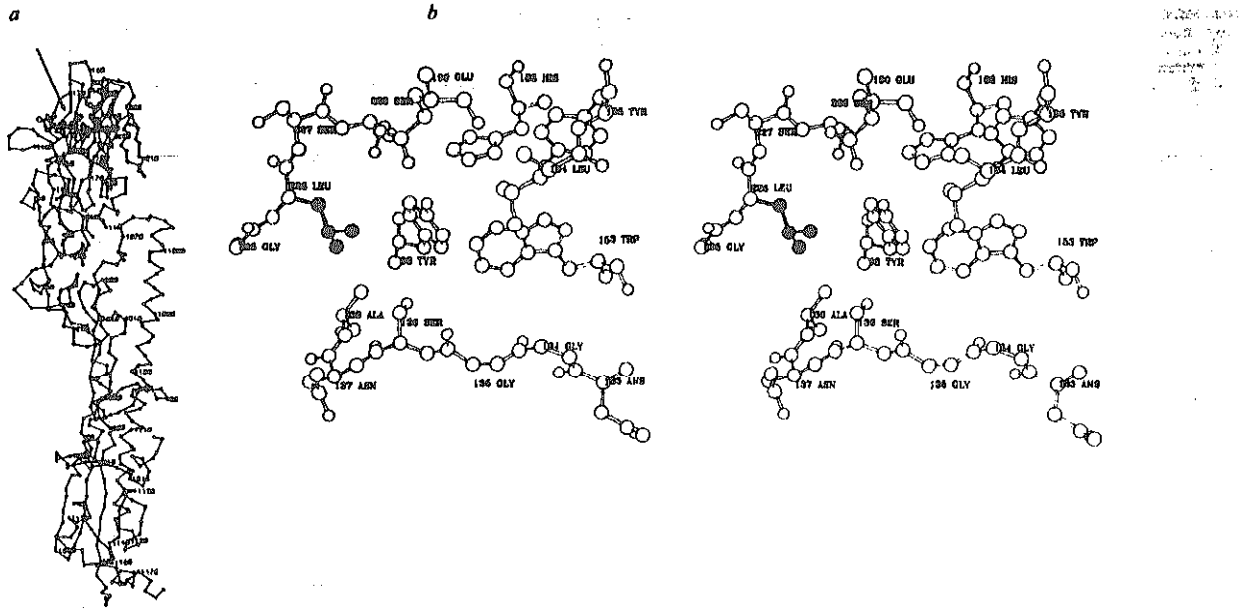

Fig. 2 *a*, Selected amino acid residues in and around the sialic acid receptor binding pocket are displayed on a schematic drawing of the α-C backbone of the haemagglutinin from the 1968 H3 influenza virus X-31. *b*, A detail of the same residues shown in *a* viewed from the top of the molecule. Leu 226 projects into the pocket near the central residue Tyr 98. The site illustrated recognizes NeuAcα2→6Gal- linkages while glutamine at position 226 alters the site to bind specifically NeuAcα2→3Gal- linkages.

specifically derivatized to contain the NeuAcα2→6Gal- and NeuAcα2→3Gal- linkages. While X-31 preferentially bound the NeuAcα2→6Gal- linkage and was very sensitive to inhibition of haemagglutination by horse serum, the selected variants bound the NeuAcα2→3Gal- linkage and were insensitive to haemagglutination inhibition.

In addition to these viruses, receptor-specific variants of A/Memphis/102/72 were obtained after growth of virus in MDCK cells in the presence and absence of horse serum and subsequent plaque purification. As indicated by the examples shown in Table 1, the viruses exhibiting preferential binding to the NeuAcα2→6Gal- linkage were inhibitor sensitive and those binding the NeuAcα2→3Gal- linkage were not. One variant which bound well to both linkages exhibited inhibitor sensitivity intermediate between the two extremes (Table 1).

The amino acid sequences of the haemagglutinins of these viruses were deduced from the nucleotide sequences of their RNA genes. These were determined using dideoxynucleotide chain terminators[8] in a primer extension system containing total virus RNA mixtures, reverse transcriptase and 5′-^{32}P-labelled synthetic oligodeoxynucleotide primers[9]. The complete sequences of the HA1 region of the haemagglutinin genes were determined for the seven viruses described in Table 1. The only amino acid sequence changes detected were at residue 226 as a result of base changes in the triplet nucleotides 754–756, CTG–leucine to CAG–glutamine or ATG–methionine (Fig. 1; see legend for discussion of an anomaly at nucleotide 742). As summarized in Table 1, amino acid 226 was leucine in viruses which preferentially bound the NeuAcα2→6Gal- linkage, glutamine for those that bound the NeuAcα2→3Gal- linkage and methionine for the variant that bound both linkages.

Examination of published amino acid sequences for the haemagglutinins of 22 influenza viruses of the H1, H2 and H3 subtypes indicates that leucine and glutamine are the only amino

acids reported for residues equivalent to 226 of haemagglutinins of the H3 subtype. This does not necessarily imply that, for example, glutamine at 226 will always specify NeuAcα2→3Gal- recognition because other amino acid changes in the site may also alter its specificity. We have, however, analysed several antigenically different H3 viruses—for example, Aichi/2/68, A/Victoria/3/75, A/Texas/1/77 and A/Duck/Ukraine/63—and in each case the observed binding specificity corresponded to that predicted by the presence of leucine or glutamine at 226[10–15] and the information presented in Table 1. Thus, despite a large background of amino acid variation observed in these haemagglutinins it is likely that the change at 226 accounts for the variations in their receptor specificity.

These observations on the modifications of sialic acid binding specificity as a consequence of amino acid substitutions at residue 226 directly support the tentative identification[5] of the sialic acid binding site as a surface pocket at the distal end of the haemagglutinin molecule. This is illustrated in the α-carbon tracing of the X-31 haemagglutinin monomer shown in Fig. 2*a*. The proposal was originally based on the presence of conserved Tyr 98, His 183, Gln 190, Trp 153 and Leu 194, amino acid residues in this pocket. Other conserved residues seem to stabilize the structure of the pocket without being in positions to interact with a receptor. Amino acid 226 is in this site (Fig. 2*b*), which is consistent with a direct role for this residue in receptor binding. We will not propose here a structurally detailed explanation for the differences in binding specificity as a result of the amino acid substitutions of glutamine and methionine for leucine because studies are in progress on crystals of haemagglutinins with different binding specificities and of haemagglutinin–sialyllactose complexes. Such studies of how single amino acid substitutions can change the specificity of haemagglutinin–sialic acid interactions may contribute to an understanding of the molecular bases of active site and receptor site binding specificities in general.

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Internal deletion in a collagen gene in a perinatal lethal form of osteogenesis imperfecta

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Cloned probes specific for unique genes have proven to be powerful tools in defining the nature of genetic diseases such as the thalassaemias[1] and growth hormone deficiencies[2]. A similar approach should be useful in defining heritable diseases of type I collagen, the heterotrimer of two α1(I) chains and one α2(I) chain, which is the most abundant member of the collagen family of proteins. Recently, cloned cDNAs and genomic DNAs for the two polypeptide chains of the type I collagen[3–10] have become available and have been used to elucidate the chromosomal location of the corresponding genes[11–14]. Here, we have used several of these cloned DNAs to demonstrate the presence of an internal deletion of about 0.5 kilobases (kb) in one allele for the proα1(I) chain in a patient with osteogenesis imperfecta (OI), a group of heritable disorders which are characterized by brittle bones but which are highly heterogeneous both phenotypically and biochemically[15,16].

The patient had a severe form of OI which was lethal perinatally[17]. Cultured fibroblasts from the patient (ATCC, CRL 1262) were initially shown[17] to secrete decreased amounts of type I procollagen, the large protein precursor of type I collagen. More recently, the patient's fibroblasts were shown to synthesize equal amounts of normal-length and shortened proα1(I) chains[18,19]. The shortened proα1(I) chains are incorporated into trimers of type I procollagen, but trimers containing the shortened proα1(I) chains have a decreased thermal stability and are rapidly degraded in fibroblast cultures[19]. The net effect is a marked reduction in the amount of type I procollagen available for fibre formation.

Total poly(A)$^+$ RNA from CRL 1262 fibroblasts was translated in a rabbit reticulocyte cell-free system[20]. The translation products contained normal-length and shortened proα1(I) chains in about equal amounts in addition to normal-length proα2(I) chains (Fig. 1). We then analysed the DNA of CRL 1262 fibroblasts by Southern blotting hybridization[21]. When a 1.8-kb cDNA covering the 3′-third of the gene[9] (Hf-677 in Fig. 2) was used as a probe, the *Hin*dIII fragment detected was identical in size to that obtained with control DNA (Fig. 3*a*2). When a 1.8-kb cDNA covering the middle of the α1(I) domain[9] (Hf-404 in Fig. 2) was used as a probe, a shortened *Hin*dIII fragment of 8.5 kb was seen in addition to the normal fragments of 6 and 9 kb (Fig. 3*a*1). The results therefore suggested a deletion of 0.5 kb within the 9-kb *Hin*dIII fragment (Fig. 2). To explore this observation further, genomic DNAs from the CRL 1262 and control fibroblasts were digested with *Bam*HI and hybridized with three genomic subclones covering the same region (M,-L.C. *et al.*, in preparation). The 5′-most probe (2.2-kb *Bam*HI subclone in Fig. 2) showed no difference from the control (Fig. 3*b*3). The middle probe (2.0-kb *Bam*HI subclone in Fig. 2) revealed an additional 6.5-kb band in the DNA from the OI fibroblasts (Fig. 3*b*4). The additional 6.5-kb band was also seen with the 3′-most probe (2-kb *Bgl*II/*Hin*dIII subclone in Fig. 2) in the OI DNA (Fig. 3*b*5). The same 3′-probe hybrydized to the short 8.5-kb band in *Hin*dIII digests of the OI DNA (Fig. 3*c*6). The data therefore indicated that one of the patient's alleles for proα1(I) contained a deletion of about 0.5 kb. The deletion eliminated the *Bam*HI site which is adjacent to an *Xho*I site found in both the gene and the cDNA and which includes the codon for amino acid residue 293 of the