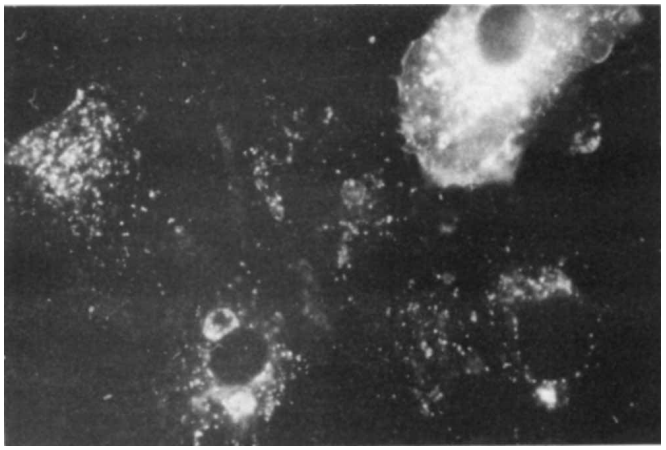

Fig. 4 Immunofluorescence micrograph of a 7-day-old thyroid monolayer fixed with acetone and stained with mouse monoclonal anti-HLA-DR as described before, showing the presence of DR antigens in cytoplasmic vesicles scattered around the nucleus. ×300.

observed only exceptionally outside the immune system[14]; guinea pig mammary gland duct epithelium becomes Ia-positive during pregnancy[15] and lactation. Ia antigen expression can be induced during graft-versus-host disease[16] and in some neoplastic conditions[17]. In these cases an immunological role has been postulated for the Ia^+ cells.

The inducibility of DR expression on thyroid cells is most remarkable in view of autoimmune phenomena affecting this gland. It is now reasonable to postulate that as yet unidentified factors may induce DR expression on thyroid cells *in vivo*. This may be an important initial or additional event by which the presentation of the relevant autoantigens to already programmed autoreactive T helper cells could lead to an effective autoimmune response. Work is in progress to determine whether a similar phenomenon can be demonstrated in other endocrine cells often affected by autoimmune reactions.

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Multiple organ-reactive monoclonal autoantibodies

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Autoantibodies directed against a wide range of normal tissue antigens have been found in the sera of patients with autoimmune diseases[1–8]. It is generally thought that different and specific autoantibodies react with different tissues but the possibility exists that some autoantibodies may react with common antigens found in different tissues and organs. Recently, we showed that mice infected with reovirus developed a polyendocrine disease with autoantibodies to the pancreas, anterior pituitary, thymus and gastric mucosa[9,10]. Using hybridoma technology, we obtained a number of monoclonal autoantibodies[11] which reacted with antigens in single organs. We now report the production and pattern of reactivity of seven multiple organ-reactive monoclonal autoantibodies. By using antibody-affinity columns, autoantigens also have been isloated and their molecular weights determined. The results suggest that monoclonal multiple organ-reactive autoantibodies react either with the same molecule present in several organs or with common antigenic determinants on different molecules in multiple organs. In either case, the existence of multiple organ-reactive antibodies may be a partial explanation for multiple organ autoimmunity.

Spleen cells from SJL/J mice infected with reovirus type 1 were fused with P3-653Ag8 myeloma cells; the culture fluids were screened by indirect immunofluorescence (FA) for antibodies reactive with paraffin sections of Bouin's-fixed normal mouse tissues (for example, adrenal glands, brain, muscle, pancreas, pituitary, salivary glands, skin, stomach, intestine, thymus, liver and thyroid), as previously described[11]. Hybrids producing autoantibodies were immediately cloned by the limiting dilution method[12]. Several days after cloning, each well was microscopically examined to ensure that it contained only a single colony. In addition, when two of the antibodies (MOR-1 and MOR-4) were subjected to isoelectric focusing on gels with a *p*H gradient of 3.5–9.5, they showed a restricted pattern typical of monoclonal antibodies[13]. Seven stable hybridomas that synthesize multiple organ reactive autoantibodes were isolated from five different animals. The reactivity pattern of these antibodies was determined by indirect immunofluorescence and/or by indirect immunoperoxidase staining using paraffin sections of normal mouse tissues fixed in Bouin's solution. The two methods gave very similar results, but the latter was generally more useful in visualizing structural details and identifying certain cell types[14].

Figure 1 shows the reactivity pattern of one of these autoantibodies (MOR-1). This antibody reacts with cells in the pituitary, pancreas, small intestine and stomach. When incubated with sections of pituitary, MOR-1 stains cells in the anterior, but not the posterior of intermediate lobes of the pituitary (Fig. 1*a*,*b*). The identity of cells involved is not known, but they do not appear to be growth hormone- or prolactin-producting cells as evaluated by staining with ffuorescein-labelled antibodies to these hormones. MOR-1 also stains cells in pancreatic islets, but not cells in pancreatic acinar tissue

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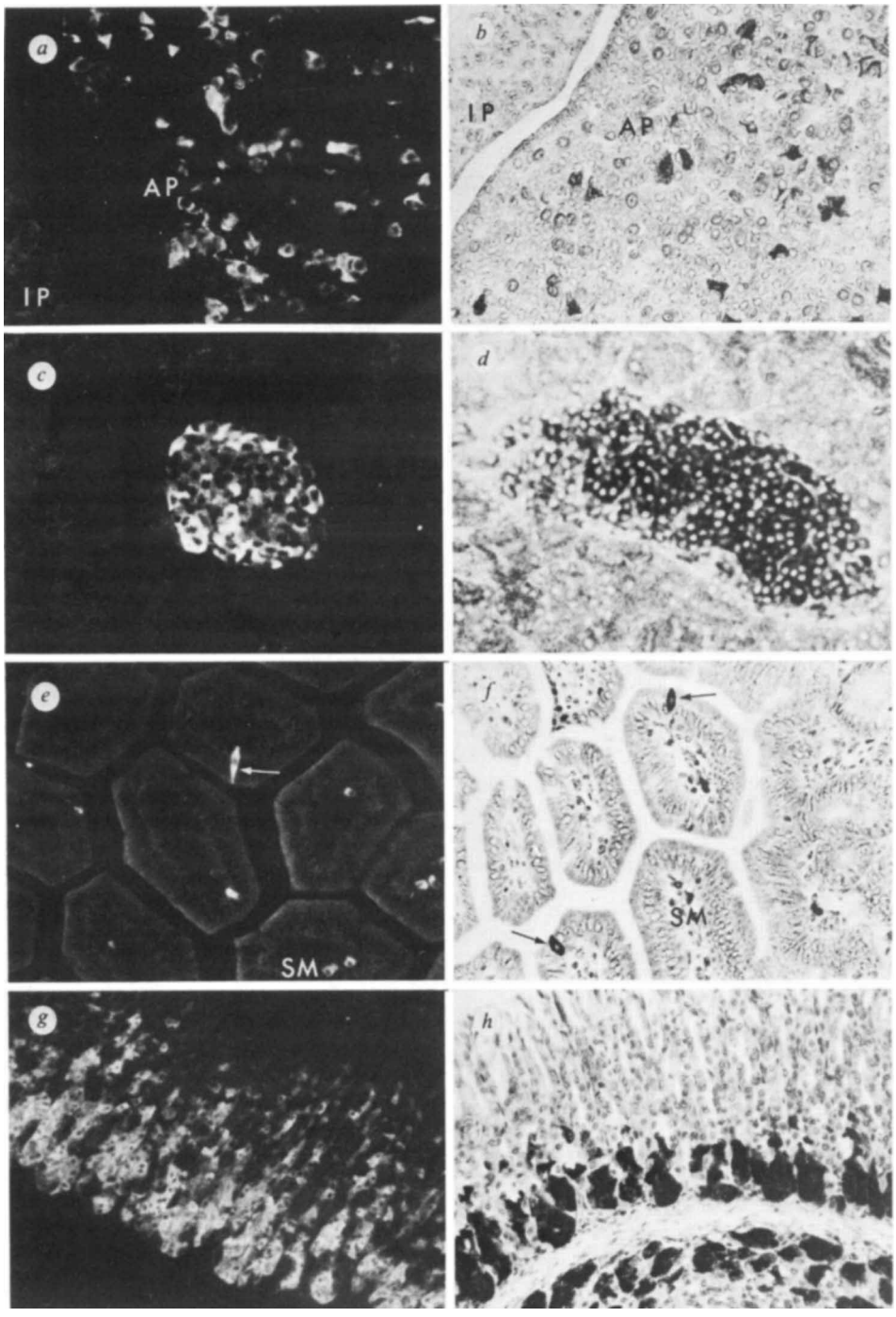

Fig. 1 Monoclonal autoantibody MOR-1 reacts with multiple organs. Normal mouse pituitary, pancreas, small intestine and stomach were fixed for 6 h in freshly prepared Bouin's solution. The specimens were washed in six changes of 70% ethanol over 72 h, embedded in paraffin and sectioned. The deparaffinized sections were first incubated with monoclonal autoantibody MOR-1 and then with affinity purified goat antibody to mouse IgM conjugated with either fluorescein isothiocyanate for indirect immunofluorescence[9] or peroxidase for indirect immunoperoxidase[13]. Panel on left stained by immunofluorescence; panel on right by immunoperoxidase. *a,b*, Sections of pituitary. Cells in the anterior pituitary (AP) stanined with MOR-1. There is no staining of cells in the intermediate (IP) or posterior lobes of the pituitary. Magnifications: *a*, ×340; *b*, ×230. *c,d*, Sections of mouse pancreas showing islet cells, but not acinar cells, stained with MOR-1. Magnifications: *c*, ×340; *d*, ×230. *e,f*, Sections of small intestine. Single isolated cells scattered throughout the small intestine (arrows) stained with MOR-1. The small labelled cells within the submucosa (SM) are most probably IgM containing plasma cells. Magnifications: *e*, ×165; *f*, ×140. *g,h*, Sections of stomach. Population of cells within the gastric mucosa stained with MOR-1. Magnifications: *g*, ×165; *h*, ×150.

(Fig. 1*c,d*). This antibody does not react with glucagon, insulin or growth hormone in enzyme-linked immunosorbent assays (ELISA) (data not shown). MOR-1 also stains single isolated basal granulated cells scattered throughout the small intestine. These cells morphologically resemble enteroendocrine cells (Fig. 1*e,f*). By staining consecutive sections, we observed that these cells are distinct from those reacting with antisera to glucagon, enkephalin and somatostatin (data not shown). MOR-1 also reacts strongly with cells in the gastric mucosa (Fig. 1*g,h*). The stained cells have been identified as chief cells by the presence of pepsinogen granules[15], but absorption of MOR-1 with porcine pepsinogen failed to block the specific labelling of gastric mucosa cells (data not shown).

The reactivity pattern of the seven multiple organ-reactive antibodies is shown in Table 1. MOR-1 reacts with cells in the gastric mucosa, anterior pituitary, small intestine and pancreatic islets; MOR-2–7 do not react with cells in the small intestines, and MOR-3 and -4 do not react with cells in the pancreatic islets. Considerable differences in the relative FA titres were observed, especially when reactivity with gastric mucosa and anterior pituitary was compared. None of the seven antibodies reacted with thyroid, adrenals, salivary glands, liver, thymus, brain, muscle or cultured mouse embryo fibroblasts (data not shown).

To isolate the autoantigens that react with multiple organ-reactive antibodies, homogenates of whole stomach (St) or pituitary (Pt) were passed through multiple organ-reactive antibody-affinity columns. Antigens St-1 and Pt-1 were eluted from an MOR-1 affinity column and antigen St-4 from an MOR-4 affinity column. These antigens were concentrated and tested on nitrocellulose paper for their reactivity with all seven MOR antibodies. As seen in Table 1, MOR-1 reacted strongly with St-1 and Pt-1, but not with St-4. Conversely, MOR-4 reacted strongly with St-4, but not with St-1 or Pt-1. MOR-2 showed

Table 1 Reactivity pattern of multiple organ-reactive monoclonal autoantibodies

Monoclonal autoantibody	Animal	Fluorescent antibody titre				Reactivity with affinity purified autoantigens		
		Gastric musosa	Anterior pituitary	Small intestine	Pancreatic islets	St-1	Pt-1	St-4
MOR-1	5B	800	800	400	40	+	+	−
MOR-2	12D	100	50	<2	10	±	±	−
MOR-3	13D	400	50	<2	<2	−	−	−
MOR-4	13D	100	100	<2	<2	−	−	+
MOR-5	13E	100	10	<2	10	−	−	−
MOR-6	13E	400	400	<2	20	−	−	−
MOR-7	13F	100	400	<2	10	−	−	−
AP-2	5B	<2	800	ND	<2	ND	ND	ND
GM-1	5A	400	<2	<2	<2	±	−	−

Monoclonal autoantibodies, precipitated from supernatant fluids by 50% saturated ammonium sulphate, were tested for reactivity with normal mouse tissues. Bouin's-fixed paraffin sections were incubated with serial dilutions of the autoantibodies followed by incubation with affinity-purified goat antibody to mouse IgG and IgM conjugated with fluorescein isothiocyanate. The fluorescent antibody titre is the reciprocal of the highest dilution giving positive fluorescence. The immunoglobulin class was determined by Ouchterlony double immunodiffusion. All the antibodies were of the IgM class, except MOR-4 which is IgG2b. To isolate the autoantigens, stomachs or pituitaries from SJL/J mice were minced and washed repeatedly in 0.1 M Tris-HCl containing 0.15 M NaCl (*p*H 7.8). Following Dounce homogenization, the homogenates were mixed with an equal volume of extraction buffer (0.1 M Tris-HCl, 0.15 M NaCl, 2% NP40) (*p*H 7.8), rehomogenized and clarified at 100,000*g* for 60 min. The supernatants thus obtained were filtered (0.2 μm) and passed through a column containing affinity purified monoclonal MOR-1 or MOR-4 autoantibodies covalently linked to cyanogen bromide-activated Sepharose CL-4B. The molecules bound to the monoclonal antibodies were eluted with either 50% ethylene glycol (*p*H 11.5) (that is MOR-1) or 1 M acetic acid (that is, MOR-4). The eluted material (St-1 and Pt-1 from the MOR-1 column and St-4 from the MOR-4 column) was dialyzed against distilled water and lyophilized. The reconstituted eluate was spotted onto nitrocellulose paper, allowed to dry and then soaked in 3% bovine serum albumin. Following overnight incubation at 4 °C with monoclonal autoantibodies, the spots were incubated for 2 h at room temperature with affinity-purified goat antibody to mouse IgG and IgM conjugated with peroxidase and then reacted with H_2O_2 and diaminobenzidine. The nitrocellulose blots were then examined for brown reaction product. (+) Strongly positive; (±) weakly positive; (−) negative.

weak reactivity with both St-1 and Pt-1. All the other antibodies failed to react with St-1, Pt-1 or St-4. Similar results, especially with MOR-1 and MOR-4, were obtained in an ELISA assay in which the antigens were adsorbed onto microtitre plates and incubated with the seven antibodies (data not shown). Taken together, these experiments show that although these antibodies react by immunofluorescence with cells in the gastric mucosa and anterior pituitary, they are not all recognizing the same antigenic determinants.

To further characterize the autoantigens, concentrated stomach and pituitary eluates from an MOR-4 affinity column were electrophoresed in SDS-polyacrylamide gels (PAGE). Four major bands (molecular weights 25,000 (25K), 43K, 53K and 62K) were obtained with the stomach eluate, and one major band (~25K) with the anterior pituitary eluate (Fig. 2*a*,*b*). The latter band consistently showed a slightly faster mobility than the 25K band from the stomach. When the gels of stomach and pituitary antigens were blotted on nitrocellulose paper[16,17], MOR-4 reacted with only three (25K, 43K, 53K) of the major stomach bands and the major pituitary band (Fig. 2*c*,*d*).

The present work demonstrates that monoclonal autoantibodies can react with antigens in multiple organs. The most likely explanation is that monoclonal autoantibodies are reacting with the same molecule present in several different tissues, and/or that they are reacting with common antigenic determinants on different molecules in multiple organs. Both explanations will probably be found to be true[18]. Our data (Fig. 2) also suggest that the multiple organ-reactive antibodies may recognize common determinants on different molecules even within the same organ. Although the autoantigens have not been fully characterized, the fact that multiple organ-reactive autoantibodies react with only certain mouse organs and not with cultured mouse cells (unpublished data) argues that these autoantibodies are not directed against easily recognized cytoskeletal elements[19].

Our studies also suggest that such antibodies are not uncommon. Although initially only seven hybridomas that synthesized multiple organ-reactive autoantibodies were found, a much larger number have recently been identified by extending our screening procedure to include more organs. There also appears to be considerable diversity in the antigenic determinants recognized. Thus, different animals, and in some cases, the same animal, make different autoantibodies to antigens in the same organ. Experiments in progress suggest that some hybridomas obtained from the spleens of normal mice also may synthesize multiple organ-reactive antibodies.

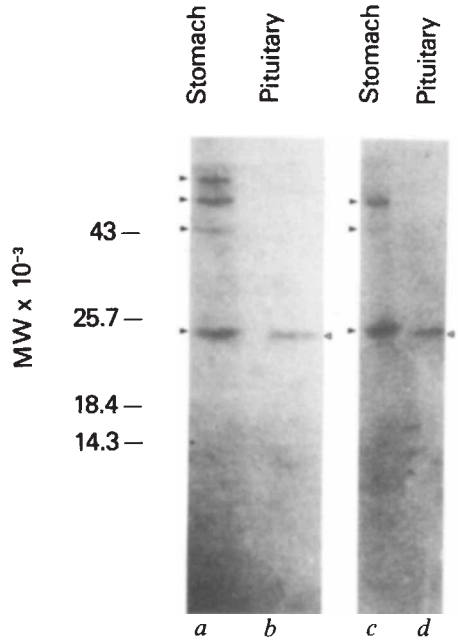

Fig. 2 Reactivity of autoantigens with MOR-4 antibody. Antigens from pituitary and stomach were isolated by affinity chromatography with MOR-4 antibody as described in the legend to Table 1, electrophoresed on 10% SDS–PAGE and blotted onto nitrocellulose paper[16,17]. Lanes *a* and *b* show the Coomassie blue staining of affinity purified stomach and pituitary antigens. Lanes *c* and *d* show the Western blots of antigens isolated from stomach and pituitary stained with MOR-4 antibody by the immunoperoxidase method. Solid and open arrows indicate polypeptides isolated from stomach and pituitary, respectively. Numbers represent molecular weight markers.

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].

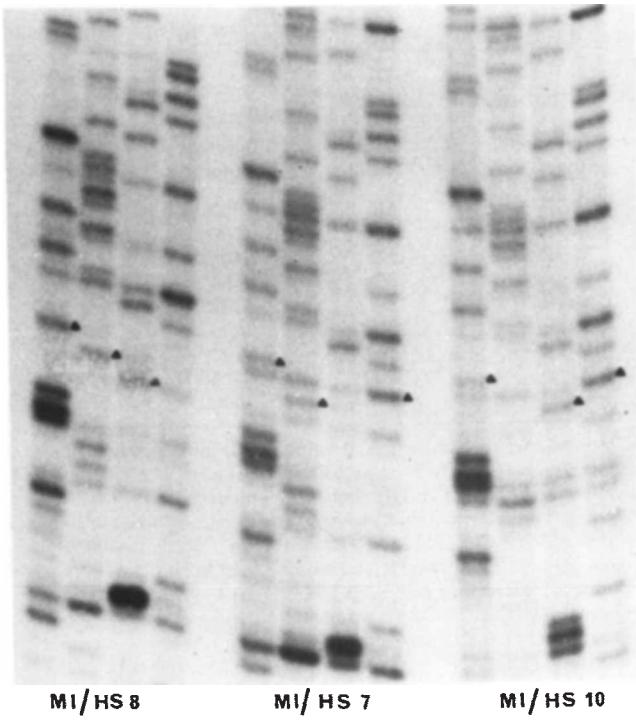

Fig. 1 Nucleotide sequence changes in the RNA of receptor binding variants. Sequences were determined using the dideoxynucleotide chain terminating procedure[8]. Each reaction mixture, 10 μl, contained Tris-Cl *p*H 8.3, 0.05 M; magnesium chloride, 0.012 M; dithiothreitol, 0.02 M; dATP, dCTP, dGTP, dTTP, 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

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