SPONTANEOUS MUTATION IN TISSUE CULTURE – CHEMICAL NATURE OF VARIANT IMMUNOGLOBULIN FROM MUTANT CLONES OF MOPC 21

D.S. SECHER*, R.G.H. COTTON** and C. MILSTEIN
Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England

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1. Introduction

Studies of spontaneous mutants occuring in cell cultures are of considerable interest for the understanding of the nature of somatic mutation in higher organisms. An ideal system for studies of this type would be one in which mutations result in a product which has no effect on the growth of the cell. This condition is approached in a myeloma cell culture, where the secreted immunoglobulin (Ig) confers no apparent benefit on the cell that produced it. Thus many mutations affecting the Ig should be tolerated, and indeed a number of variants with defects in Ig synthesis have been described [1]. However, none of these, nor any other somatic mutation in higher animal cells, has been shown to be the result of a mutation in the structural gene coding for the variant protein.

We have described a novel technique for the screening of large numbers of clones from a myeloma cell culture for electrophoretic variants of the secreted Ig [2]. The method allowed detection of clones deficient in Ig production and also of a clone that secretes an Ig of altered isoelectric point (IF-1). A second clone producing a variant IgG (IF-2) has now been isolated. We describe here experiments that indicate that these mutants arise as a result of mutations in Ig structural genes.

2. Cell lines and methods

All the lines used are derived from the mouse plasma cell tumour (MOPC 21) adapted to grow in tissue culture (P3K) [3]. P3/X27 is a wild-type clone of P3K.

The maintenance of cell lines, the method of screening and the examination of intercellular material have been described [2]. IF-1 was also maintained as solid tumours by subcutaneous injection of IF-1 cells into Balb/c mice and serial passage of the tumours that develop. Myeloma protein was prepared from the serum of tumour-bearing animals by ammonium sulphate precipitation and DEAE-cellulose ion-exchange chromatography [4]. [^{14}C]lysine-, [^{14}C]arginine- and [^{35}S]cystine-labelled proteins were prepared essentially according to Svasti and Milstein [4]. Sodium dodecyl sulphate (SDS)-gel electrophoresis was performed in 12% polyacrylamide slabs using a discontinuous buffer system [5].

Partially purified mRNA from 7×10^8 IF-1 tissue culture cells was obtained by fractionation of microsomal RNA on SDS-sucrose gradients [6]. RNA isolated from fractions taken from such gradients was assayed for mRNA activity in a rabbit reticulocyte cell-free system [7] by incorporation of [^{35}S]methionine followed by SDS-gel electrophoresis of the total incubation mixture.

3. Results

Screening of 1140 clones from a P3K culture that was about 450 generations old revealed a variant clone (IF-2) that secretes a product of lower isoelectric point than the IgG of the wild-type (P3K) line from which it was derived (fig. 1b, c). The cellular changes which result in the production of a variant Ig appear to be

*M.R.C. scholar

**Present address: Royal Children's Hospital Research Foundation, Flemington Road, Parkville, Victoria, Australia.

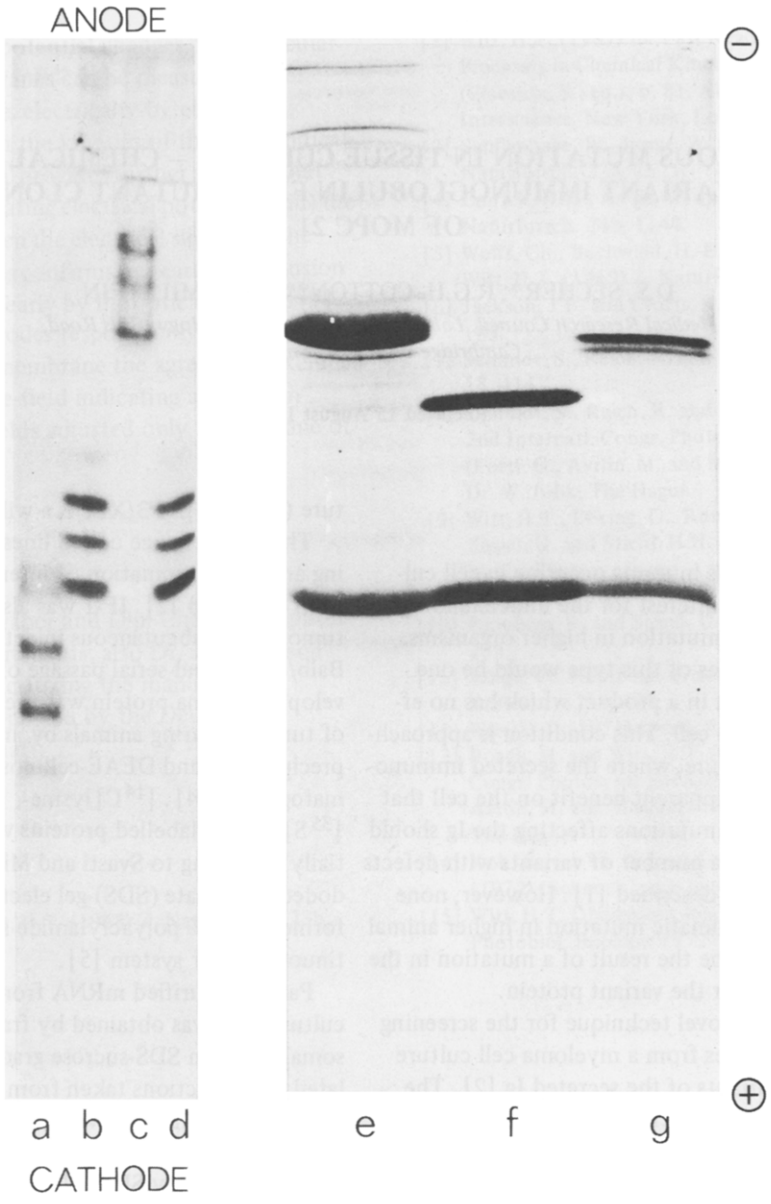

Fig. 1. Comparison of ^{14}C-labelled Ig of wild type and mutant clones by isoelectric focusing in the range pH 6–8 (a–d) and by SDS-gel electrophoresis of the reduced molecules (e–g): a, g, IF-1; b, d, e, P3/X27; c, f, IF-2.

stable since IF-1 and IF-2 have been maintained in continuous culture for several months without showing any tendency to revert to P3K IgG production.

Cellulose acetate electrophoresis indicated that the major labelled products secreted by IF-1 and IF-2 had the electrophoretic mobility of γ-globulins. Both proteins were purified using a standard procedure for IgG purification [4]. SDS-gel electrophoresis showed that IF-1 and IF-2 proteins both contain light (L)-chains of normal size, whereas the heavy (H)-chains are different from P3K H-chain (fig. 1e, f, g). Tryptic fingerprints (to be published elsewhere in detail) did not reveal differences between the three L-chains. By contrast the H-chain fingerprints showed clear differences,

although most of the spots were common to all three digests. Analysis of the peptides and comparison with known sequences in P3K H-chain [4] or with the complete sequence of a human H-chain [8] allowed the assignment of many spots to known regions of the H-chain.

3.1. *IF-1 heavy-chain*

By SDS-gel electrophoresis, the size (mobility) difference between IF-1 and wild-type H-chain is small.

We have previously shown that IF-1 Ig contains sialic acid while P3K Ig does not [9]. To investigate the possibility that this difference in carbohydrate content could account for the differences between the H-chains of IF-1 and wild-type protein, intracellular Ig was subjected to isoelectric focusing following a 15 min pulse of [^{14}C]lysine. Radioautography of the gel revealed clear differences between the products of P3K and IF-1 cells (fig. 2). In IF-1 the most prominent band (characteristic of newly synthesised protein) had a higher isoelectric point than that of P3/X27. (Neither of these bands was affected by treatment with neuraminidase.) Thus the difference between IF-1 and P3/X27 IgG, already apparent in intracellular material, does not seem to be related to changes in either the secretion process or other post-translational events. To investigate differences between P3K and IF-1 H-chain mRNA's the synthesis of H-chains [10] in a heterologous (reticulocyte lysate) cell-free system was examined.

RNA fractions from the 12–20 S region of an SDS-sucrose density gradient were observed to stimulate the incorporation of [^{35}S]methionine into H- and L-chains (fig. 3). As expected the major product of synthesis directed by the IF-1 mRNA of lower molecular weight co-electrophoresed with the translation product of P3/X27 L-chain mRNA (fig. 3, band P). However, the protein synthesised on addition of IF-1 mRNA of higher molecular weight (from about 18 S) was markedly different from P3/X27 H-chain (fig. 3b, c).

These results indicate that the mRNA's coding for IF-1 and P3K H-chains are different and that the mutation involves an Ig structural gene. As a consequence of the mutation, IF-1 H-chain appears to be smaller than P3K H-chain. The site of the apparent deletion in the IF-1 H-chain was investigated by N- and C-terminal analysis and by examination of the tryptic fingerprints.

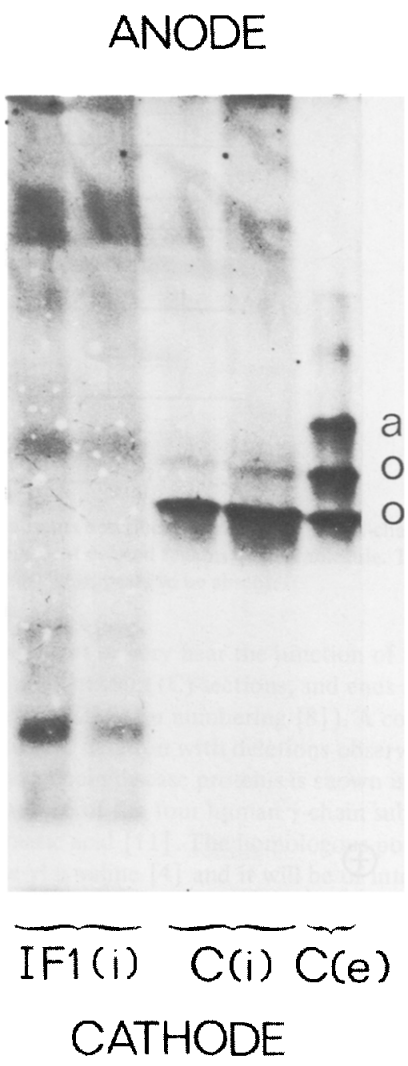

Fig. 2. Intracellular material from IF-1 (IF1 (i)) and P3/X27 (C (i)) labelled in a 15 min incubation of cells in medium containing [^{14}C]lysine [2]. Samples were analysed by isoelectric focusing (pH 6–8) and autoradiography of the dried gel. C (e) is a sample of secreted Ig from P3/X27 shown for comparison. o, o', a, refer to the nomenclature of Awdeh et al. [13].

Sequentator analysis showed that the first eight amino-terminal residues of IF-1 and P3K H-chains are identical. Carboxypeptidase A released valine from IF-1 H-chain, but no free amino acid was detected in a control using the P3K H-chain. These results suggest that IF-1 H-chain is a consequence of a deletion at the C-terminus, exposing valine as the new

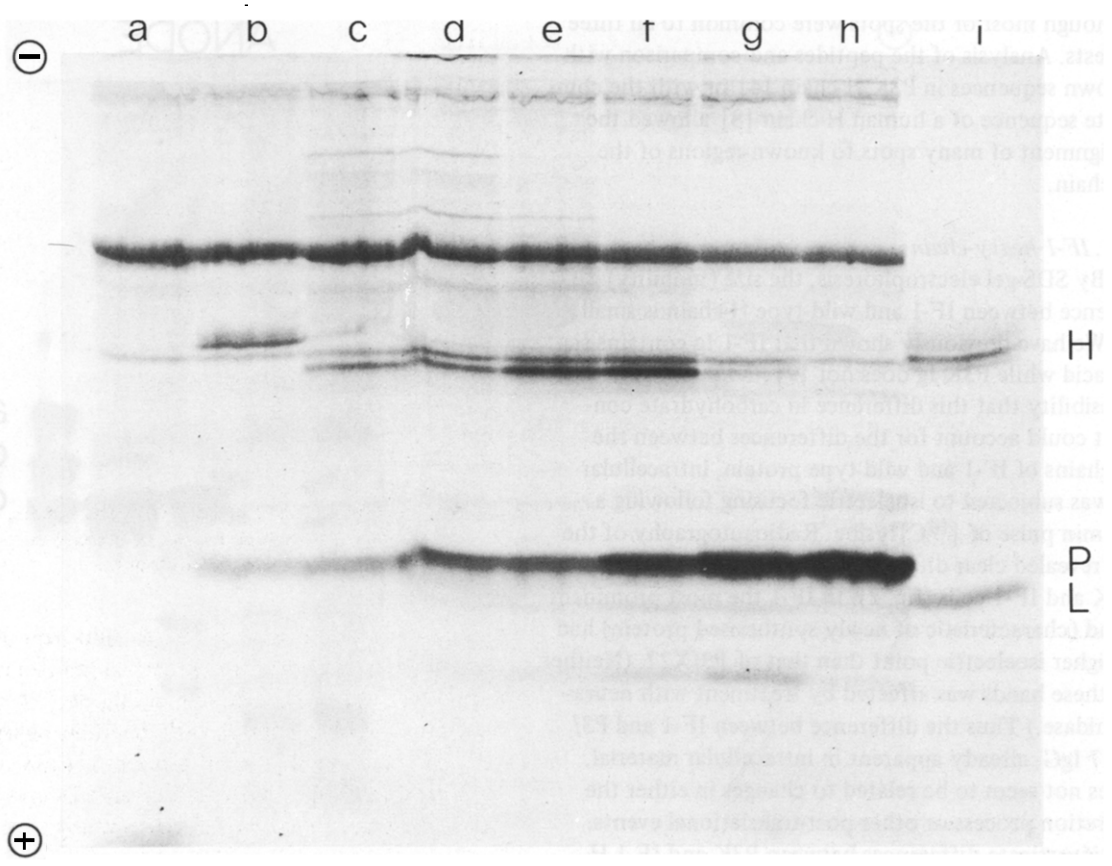

Fig. 3. Labelled products obtained by SDS-gel electrophoresis of rabbit reticulocyte lysates incubated with [^{35}S]methionine. RNA was added to the incubations as follows: a, no RNA added; b, P3/X27 H- and L-chain mRNA's; c–h, IF-1 microsomal RNA of decreasing sedimentation coefficient from about 20 S to 10 S isolated from consecutive fractions of an SDS-sucrose density gradient; i, a mixture of ^{14}C-labelled IgG secreted by P3/X27 and IF-1. Band P is the L-chain Precursor [14].

C-terminal residue. This interpretation was supported by the tryptic fingerprints. Some peptides common to IF-1 and P3K H-chains were identified as originating from the N-terminal region of the H-chain, whereas spots absent from IF-1 but present in P3/X27 H-chain fingerprints could be assigned to peptides close to the C-terminus.

3.2. *IF-2 heavy-chain*

SDS-gel electrophoresis showed a large mobility difference between H-chains of IF-2 and P3K, suggesting that IF-2 might also arise as the result of a deletion. In this case examination of tryptic fingerprints of H-chains labelled with [^{14}C]lysine and [^{14}C]arginine revealed peptides from N- and C-terminal regions common to P3/X27 and IF-2, whereas spots corresponding to peptides near the hinge-region of P3/X27 H-chain were absent in the IF-2 fingerprint. Striking differences were observed in fingerprints of [^{35}S]cystine-labelled proteins. The peptic peptide (PHOA) [4], which in P3/X27 contains all the inter-heavy chain disulphide bridges, is missing in IF-2 and a peptide unique to IF-2 (PH2) appears to replace it. Tryptic digestion of PHOA and PH2 produced a common peptide containing the disulphide bridges. Thus the deletion in IF-2 appears to be intramolecular, involving a region localised towards the N-terminal side of the hinge-region, which contains the inter-heavy disulphide bridges. The exact extent of the deletion is not yet known, but mobilities on

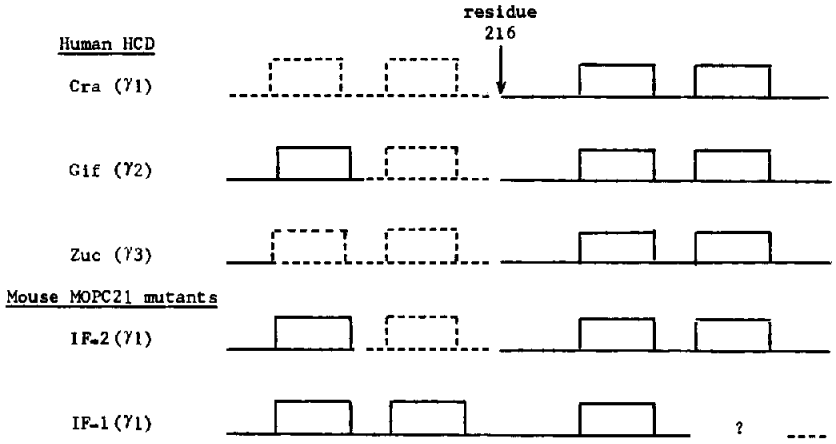

Fig. 4. A comparison of deletions in human heavy chains with those in the mouse mutants described in this paper. The H-chain is shown diagramatically with its four intrachain disulphide bridges. Broken lines represent deleted regions of the molecule. The extent of the deletion in IF-1 is not yet known, although the C-terminal disulphide bridge appears to be absent.

SDS-gels suggest that it could involve as many as 100 residues.

4. Discussion

The results presented demonstrate that mutants with alterations in their Ig structural genes can be isolated from tissue-cultured cells. It is possible that the nature of the genetic events leading to the structural alterations of IF-1 and IF-2 is different. At this stage IF-1 could be explained by a point mutation, frame shift, or out of phase recombination giving rise to a chain termination triplet. IF-2, on the other hand, appears to be the result of a recombinational event leading to an internal deletion.

The C-terminus of IF-1 differs from that of the parental H-chain, which suggests that the deletion involves the C-terminal section. Proteolysis of a C-terminal portion seems excluded in view of the results obtained on intracellular and in vitro synthesised material. The differences between the carbohydrate of wild-type and IF-1 H-chains are likely to be secondary changes due to a failure to recognise the altered Fc correctly.

The mutation observed in IF-2 involves an internal deletion of about 100 residues. Further analysis of the altered peptides will reveal the exact position of the deletion, but from the results obtained so far it is clear that it starts at or very near the junction of the variable (V)- and constant (C)-sections, and ends at or close to residue 216 (Eu numbering [8]). A comparison of the IF-2 deletion with deletions observed in human heavy chain disease proteins is shown in fig. 4. Residue 216 of the four human γ-chain subclasses is glumatic acid [11]. The homologous position in mouse γ1 is valine [4] and it will be of interest to know whether it is at this same site that mutant IF-2 and wild-type H-chains regain homology.

In the case of human heavy chain disease the presumed parental protein has never been available. In contrast, the heavy chain of IF-2 can be compared with the MOPC 21 heavy chain. Therefore one can ask whether the V-section of the deleted protein is identical to the parental one. The fingerprint evidence suggests that this is so, but confirmation by extensive sequence studies is required. If it proves to be the case it would strongly suggest that the deletion occured after integration of V- and C-genes, providing further evidence that integration occurs at the DNA level [12] The fact that in mouse, as in humans, residue 216 appears to be a particular target in deletion events remains a puzzle. It is as if that part of the DNA is somehow similar to a signal for recombinational events, making it prone to this type of mistake. Whether this signal is related to those involved in V–C integration can only be a matter of speculation at this stage.

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