LETTERS TO NATURE

Definition of 'charge on an atom' and nature of the inductive effect—S. M. Dean and W. G. Richards

A low velocity zone underlying a fast-spreading rise crest—J. Occelli, B. Kenneth, L. Dorman and W. Prothero

Mercury contamination in a 54-m core from lake Holeh—U. M. Cowgill

A resonant point absorber of oceanic wave power—K. Bader and J. Felsen


American 242Sn in nuclear test debris—V. T. Brown and H. D. Livingston

Tree remains in southern Pennine peats—J. H. Talits

Regularities in duration of regional desert locust plagues—Z. Waloff and S. M. Green

Development of a desert locust plague—L. V. Beawett

Seed-borne microorganisms stimulate seedcorn maggot egg laying—C. J. Eckenrode, G. E. Harman and D. R. Webb

Defensive stoning by baboons—W. J. Hamilton III, R. E. Backlund and W. H. Backlund

Eccentricity-specific dissociation of visual functions in patients with lesions of the central visual pathways—E. Pippel, D. von Cramon and H. Backlund

Evidence for visual function mediated by anomalous projection in goldfish—D. Jager and S. C. Shamna

Thyroid rudiment of the athmic nude mouse—M. Holdh, P. Rossman, H. Tlashakovna and H. Vidmarova

Striated muscle fibres differentiate in monolayer cultures of adult thymus reticulum—H. Wekerle, B. Paterson, U.-P. Ketelsen and M. Feldman

Continuous cultures of fused cells secreting antibody of predeftned specificity—G. Kohler and C. Milstein

Naturally occurring cytotoxic tumour reactive antibodies directed against type C viral envelope antigens—S. E. Morris and W. J. Martin

Antigen formation in metal contact sensitivity—J. M. Jones and H. E. Amos

Induced thermal resistance in HeLa cells—E. W. Garner and M. J. Schneider

Regional turnover and synthesis of catecholamines in rat hypothalamus—D. H. G. Verteer, J. van der Gietgen and J. M. van Ree

Rate of nucleohogon in as a measure of gene activity—C. de la Torre, M. E. Fernandez-Gomez and G. Jimenez-Martin

5S RNA secondary structure—G. E. Fox and C. R. Woese

Differential effect of plasma fractions from normal and tumour-bearing rats on nuclear RNA synthesis—D. E. Schumon and T. E. Webb

Early role during chemical evolution for cytochrome P450 in oxygen detoxification—R. H. Wicker, A. K. Wilson and C. A. Villee

Human embryonic haemoglobin including a comparison by homology of the humanζ and α chains—J. R. H. Kamzor and H. Lehmann

Lycorine as an inhibitor of ascorbic acid biosynthesis—O. Arrigoni, R. A. Liso and G. Calabrese

Intracellular killing of Listeria monocytogenes by activated macrophages (Mackinnon system) is due to antibiotic—P. Cole and J. Brostoff
Continuous cultures of fused cells secreting antibody of predefined specificity

The manufacture of predefined specific antibodies by means of permanent tissue culture cell lines is of general interest. There are at present a considerable number of permanent cultures of myeloma cells and screening procedures have been used to reveal antibody activity in some of them. However, this is not a satisfactory source of monoclonal antibodies of predefined specificity. We describe here the derivation of a number of tissue culture cell lines which secrete anti-sheep red blood cell (SRBC) antibodies. The cell lines are made by fusion of a mouse myeloma and mouse spleen cells from an immunised donor. To understand the expression and interactions of the Ig chains from the parental lines, fusion experiments between two known mouse myeloma lines were carried out.

Each immunoglobulin chain results from the integrated expression of one of several V and C genes coding respectively for its variable and constant sections. Each cell expresses only one of the two possible alleles (allelic exclusion; reviewed in ref. 3). When two antibody-producing cells are fused, the products of both parental lines are expressed5,6, and although the light and heavy chains of both parental lines are randomly joined, no evidence of scrambling of V and C sections is observed.4 These results, obtained in an heterologous system involving cells of rat and mouse origin, have now been confirmed by fusing two myeloma cells of the same mouse strain.

The protein secreted (MOPC 21) is an IgG1 (k) which has been fully sequenced7. Equal numbers of cells from each parental line were fused using inactivated Sendai virus8 and samples containing 2 × 10^9 cells were grown in selective medium in separate dishes. Four out of ten dishes showed growth in selective medium and these were taken as independent hybrid lines, probably derived from single fusion events. The karyotype of the hybrid cells after 3 months in culture was just under the sum of the two parental lines (Table 1). Figure 1 shows the isoelectric focusing9 (IEF) pattern of the secreted products of different lines. The hybrid cells (samples a-b in Fig. 1) give a much more complex pattern than either parent (a and b) or a mixture of the parental lines (m). The important feature of the new pattern is the presence of extra bands (Fig. 1, arrows). These new bands, however, do not seem to be the result of differences in primary structure; this is indicated by the IEF pattern of the products after reduction to separate heavy and light chains (Fig. 1B). The IEF pattern of chains of the hybrid clones (Fig. 1B, g) is equivalent to the sum of the IEF pattern (a and b) of chains of the parental clones with no evidence of extra products. We conclude that, as previously shown with interspecies hybrids5,9, new Ig molecules are produced as a result of mixed association between heavy and light chains from the two parents. This process is intracellular as a mixed cell population does not give rise to such hybrid molecules (compare m and g, Fig.1A). The individual cells must therefore be able to express both isotypes. This result shows that in hybrid cells the expression of one isotype and idiotype does not exclude the expression of another: both heavy chain isotypes (y1 and y2a) and both V, and both V, regions (idiotypes) are expressed. There are no allotypic markers for the C region to provide direct proof for the expression of both parental C regions. But this is indicated by the phenotypic link between the V and C regions.

Figure 1A shows that clones derived from different hybridisation experiments and from subclones of one line are indistinguishable. This has also been observed in other experiments (data not shown). Variants were, however, found in a survey of 100 subclones. The difference is often associated with changes...
assays have been used to detect specific clones and representative clones of both types have been characterised and studied.

The derived lines (Sp hybrids) are hybrid cell lines for the following reasons. They grow in selective medium. Their karyotype after 4 months in culture (Table 1) is a little smaller than the sum of the two parental lines but more than twice the chromosome numbers of normal BALB/c cells, indicating that the lines are not the result of fusion between spleen cells. In addition the lines contain a metacentric chromosome also present in the parental P3-X67Ag8. Finally, the secreted immunoglobulins contain MOPC 21 protein in addition to new, unknown components. The latter presumably represent the chains derived from the specific anti-SRBC antibody. Figure 3.4 shows the IEF pattern of the material secreted by two such Sp hybrid clones. The IEF bands derived from the parental P3 line are visible in the pattern of the hybrid cells, although obscured by the presence of a number of additional bands. The pattern is very complex, but the complexity of hybrids of this type is likely to result from the random recombination of chains (see above, Fig. 1). Indeed, IEF patterns of the reduced material secreted by the spleen-P3 hybrid clones gave a simpler pattern of Ig chains. The heavy and light chains of the P3 parental line became prominent, and new bands were apparent.

Hybrid Sp-I gave direct plaques and this suggested that it produces an IgM antibody. This is confirmed in Fig. 4 which shows the inhibition of SRBC lysis by a specific anti-IgM

| Table 1 Number of chromosomes in parental and hybrid cell lines |
|-------------------|---------------------|-------------------|
| Cell line         | Number of chromosomes per cell | Mean |
| P3-X67Ag8         | 66,65,65,65,65       | 65     |
| P3 Bal            | Ref. 4               | 55     |
| Mouse spleen cells| 24                   | 110    |
| Hy-B (P1-P3)      | 112,110,104,104,102  | 106    |
| Sp-1/7-2          | 93,90,89,89,87       | 90     |
| Sp-2/3-3          | 97,95,95,95,95,95,95,95,95 | 95     |

Fig. 2 Isolation of an anti-SRBC antibody secreting cell clone. Activity was revealed by a halo of haemolysed SRBC. Direct plaques were given by: a, 6,000 hybrid cells Sp-1; b, clones grown in soft agar from a suspension of 10° Sp-1 cells, c, recloning of one of the positive clones Sp-1/7; d, higher magnification of a positive clone. Myeloma cells (10° P3-X67Ag8) were fused to 10° spleen cells from an immunised BALB/c mouse. Mice were immunised by intraperitoneal injection of 0.2 ml packed SRBC diluted 1:10, boosted after 1 month and the spleens collected 4 days later. After fusion, cells (Sp-1) were grown for 4 days in HAT medium, changed to 1-3 d medium. Cells were then grown in Dulbecco modified Eagle's medium, supplemented for 2 weeks with hypoxanthine and thymidine. Forty days after fusion the presence of anti-SRBC activity was revealed as shown in a. The ratio of plaque forming cells/total number of hybrid cells was 1/30. This hybrid clone was cloned in soft agar (50% cloning efficiency). A modified plaque assay was used to reveal positive clones shown in b as follows. When cell clones had reached a suitable size, they were overlaid in sterile conditions with 2 ml 0.6% agarose in phosphate-buffered saline containing 25 μl packed SRBC and 0.2 ml fresh guinea pig serum (absorbed with SRBC) as source of complement. b, Taken after overnight incubation at 37°C. The ratio of positive/plaque number of clones was 1/33. A suitable positive clone was picked out and grown in suspension. This clone was called Sp-1/7, and was recloned as shown in c; over 90% of the clones gave positive lysis. A second experiment in which 10° P3-X67Ag8 cells were fused with 10° spleen cells was the source of a clone giving rise to indirect plaques (clone Sp-2/3-3). Indirect plaques were produced by the addition of 1:20 sheep anti-MOPC 21 antibody to the agarose overlay.

in the ratios of the different chains and occasionally with the total disappearance of one or other of the chains. Such events are best visualised on IEF analysis of the separated chains (for example, Fig. 1b, in which the heavy chain of P3 is no longer observed). The important point that no new chains are detected by IEF complements a previous study of a rat-mouse hybrid line in which scrambling of V and C regions from the light chains of rat and mouse was not observed. In this study, both light chains have identical C regions and therefore scrambled V-L-C molecules would be undetected. On the other hand, the heavy chains are of different subclasses and we expect scrambled V-H-C molecules to be detectable by IEF. They were not observed in the clones studied and if they occur must do so at a lower frequency. We conclude that in syngeneic cell hybrids (as well as in interspecies cell hybrids) V-C integration is not the result of cytoplasmic events. Integration as a result of DNA translocation or rearrangement during transcription is also suggested by the presence of integrated mRNA molecules and by the existence of defective heavy chains in which a deletion of V and C sections seems to take place in already committed cells.

The cell line P3-X67Ag8 described above dies when exposed to HAT medium. Spleen cells from an immunised mouse also die in growth medium. When both cells are fused by Sendai virus and the resulting mixture is grown in HAT medium, surviving clones can be observed to grow and become established after a few weeks. We have used SRBC as immunogen, which enabled us, after culturing the fused lines, to determine the presence of specific antibody-producing cells by a plaque assay technique (Fig. 2a). The hybrid cells were cloned in soft agar and clones producing antibody were easily detected by an overlay of SRBC and complement (Fig. 2b). Individual clones were isolated and shown to retain their phenotype as almost all the clones of the derived purified line are capable of fixing SRBC (Fig. 2c). The clones were visible to the naked eye (for example, Fig. 2d). Both indirect and direct plaque
antibody. IEF techniques usually do not reveal 19S IgM molecules. IgM is therefore unlikely to be present in the produced sample a (Fig. 3H) but μ chains should contribute the pattern obtained after reduction (sample a, Fig. 3A).

The above results show that cell fusion techniques are a powerful tool to produce specific antibody directed against a predetermined antigen. It further shows that it is possible to isolate hybrid lines producing different antibodies directed against the same antigen and carrying different effector functions (direct and indirect plaque).

The uncloned population of P3-spleen hybrid cells seems quite heterogeneous. Using suitable detection procedures it should be possible to isolate tissue culture cell lines making different classes of antibody. To facilitate our studies we have used a myeloma parental line which itself produced an Ig variants in which one of the parental chains is no longer expressed seem fairly common in the case of P1-P3 hybrids (Fig. 1H). Therefore selection of lines in which only the specific antibody chains are expressed seems reasonably simple.

Alternatively, non-producing variants of myeloma lines could be used for fusion.

We used SRBC as antigen. Three different fusion experiments were successful in producing a large number of antibody-producing cells. Three weeks after the initial fusion, 33/1,086 clones (3%) were positive by the direct plaque assay. The cloning efficiency in the experiment was 50%. In another experiment, however, the proportion of positive clones was considerably lower (about 0.2%). In a third experiment the hybrid population was studied by limiting dilution analysis. From 157 independent hybrids, as many as 15 had anti-SRBC activity. The proportion of positive over negative clones is remarkably high. It is possible that spleen cells which have been triggered during immunisation are particularly successful in giving rise to viable hybrids. It remains to be seen whether similar results can be obtained using other antigens.

The cells used in this study are all of BALB/c origin and the hybrid clones can be injected into BALB/c mice to produce solid tumours and serum having anti-SRBC activity. It is possible to hybridise antibody-producing cells from different origins. Such cells can be grown in vitro in massive cultures to provide specific antibody. Such cultures could be valuable for medical and industrial use.

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

G. Köhler
C. Milstein

Received May 14; accepted June 26, 1975.