strains, small but significant interstrain differences in lactate dehydrogenase activity were observed[11]. Therefore, it seems that biochemical data on the ova of random bred Swiss mice may not be generally representative.

We conclude that the absolute level of ova GPI activity, although strictly regulated within each strain, can vary over a considerable range and yet permit successful reproduction. This situation now provides a unique opportunity for further exploration into developmental gene regulation during oogenesis and also during preimplantation development[12,13].

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A better cell line for making hybridomas secreting specific antibodies

FUSION of myeloma cells which grow in tissue culture with spleen cells from an immunised mouse provides a general method for obtaining cell lines (hybridomas) which make antibody of the desired specificity[1–3]. Hybrids derived from these myelomas make the immunoglobulin (Ig) heavy and light chains of the myeloma parent as well as the antigen-specific heavy and light chains of the spleen cell parent. In conditions in which the two heavy and two light chains associate randomly, a hybridoma would make 10 distinct Ig molecules, and the specific antibody would comprise only 1/16 of the total Ig[4,5]. To obtain hybridomas making only the specific antibodies requires a tumour cell fusion partner that itself makes no Ig but which can nevertheless be fused with spleen cells to obtain hybrids secreting only the specific antibody. We report here the identification of such a cell line, Sp2/0-Ag14.

Sp2/0-Ag14 was isolated as a re-clone of Sp2/HL-Ag, itself derived in several steps from Sp2/HLGK, a hybrid between a BALB/c spleen cell contributing a γ2b (H) and κ (L) chain with anti-sheep red blood cell activity and the myeloma cell line X63-Ag8 (γ (G) and κ (K))[2]. Sp2/0-Ag14 is resistant to 20 μg ml^{-1} 8-azaguanine, dies in HAT supplemented medium and synthesises no Ig chains. It has about 73 chromosomes which is only eight more than the chromosome number of X63-Ag8, a cell line commonly used to generate hybridomas.

Sp2/0-Ag14 was fused with spleen cells of mice immunised with trinitrophenyl derivatised keyhole limpet haemocyanin (TNP-KLH) to generate hybridomas making TNP-specific immunoglobulin (Fig. 1). Fusions were done with either polyethylene glycol (PEG) 1500 or Sendai virus, and transferred to medium either containing or lacking mouse peritoneal exudate cells. Growth was observed in only eight culture wells, all from the set that were fused with PEG and grown with peritoneal exudate cells.

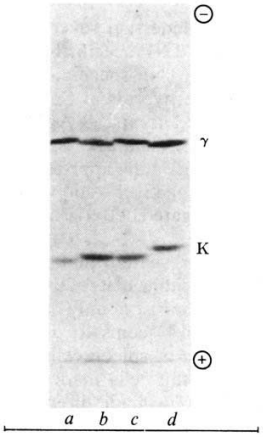

Fig. 1 Immunoglobulin from TNP-specific hybridomas analysed by SDS-polyacrylamide gel electrophoresis. To obtain hybridomas making Ig specific for the hapten trinitrophenyl, BALB/c mice were immunised i.p. with trinitrophenyl-derivitised keyhold limpet haemocyanin (TNP-KLH) (from H. Kiefer), first with 100 μg TNP-KLH contained in 0.2 ml emulsion of 50% phosphate-buffered saline (PBS), 50% complete Freund's adjuvant (Difco). 35 days later a second injection of 100 μg TNP-KLH in 0.2 ml PBS was made i.p., and 5 days later the spleens of these mice were prepared for fusion. Fusion with PEG 1500 was as described by Galfre *et al.*[9], except that the medium used was R medium, RPMI 1640 medium (Gibco) supplemented with 30mM HEPES (Flow). Here 3×10^7 Sp2/0-Ag14 and 5×10^7 spleen cells were centrifuged together at 200 g for 5 min and resuspended slowly in 0.6 ml 50% PEG in Dulbecco's modified Eagle's medium (Flow). After 1 min at 37 °C, 20 ml of R medium was added slowly. The cells were then centifuged and resuspended in 20 ml of R medium supplemented with 10% fetal calf serum (Gibco) (RF medium) and 0.2 ml of this suspension was then distributed to each of 200 wells containing 0.8 ml RF medium. One hundred of these wells also contained 2×10^5 mouse peritoneal exudate cells. After 24 h incubation, 1 ml RF supplemented with hypoxanthine, aminopterin and thymidine (HAT) was added to each well. Every 2–3 days, 1 ml of the medium was replaced with fresh RF+HAT. After 2 weeks, growth was observed in eight wells of the peritoneal exudate supplemented wells and the cultures were tested for immunoglobulin production by testing the culture medium for lysis of protein A coated sheep red blood cells (SRC) for nonspecific Ig[10] and of TNP coated SRC[2] (for TNP specific activity). After 1–2 months the cells from TNP-specific wells were cloned by plating in soft agar[11] or in methyl cellulose[12]. The soft agar clones were overlaid with TNP coated SRC, and clones lysing the TNP coated SRC were transferred to liquid medium. The cells were grown in the presence of ^{14}C leucine to label secreted Ig which was analysed by SDS polyacrylamide gel electrophoresis as described[11]. The hybridoma cell lines analysed were hy5.19 (*a*), hy2.15 (*b*), hy1.2 (*c*) and hy3.3 (*d*).

Supernatant of four of the eight growth-positive wells lysed protein A but not TNP-coated sheep red blood cells (SRC) suggesting that the Ig made by these cells did not have TNP specificity. The Ig secreted by these cells was analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and found to be IgM (data not shown). The remaining four growth positive wells lysed both TNP and protein A SRC and cells from these wells were cloned and analysed further, as described below. SDS-PAGE analysis suggested that these cell lines secreted IgG (Fig. 1). Ouchterlony analysis of culture supernatants using class-specific antisera (Litton Bionetics) indicated that hyl.2 makes an IgG2a, and that hy2.15, hy3.3 and hy5.19 make IgGl and that all four TNP-specific hybridomas make a κ light chain. The SDS-PAGE analysis shown in Fig. 1 indicates that the clones can be distinguished by the mobility of the light or heavy chain, implying that these cell lines are making different antibodies.

We have examined the TNP-specific hybridomas for re-expression of the SRC-specific IgG2b of the progenitor cell line Sp2/HL-Ag14 by testing for SRC-specific plaques. No plaques

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were found and we conclude that revertants constitute fewer than one in 5×10^4 of the TNP-specific hybridoma cells.

The karyotypes of the TNP-specific cell lines provide evidence that these cells are hybrids of Sp2/0-Ag14 and mouse cells. Normal mouse cells and Sp2/0-Ag14 contain 40 and 73 chromosomes, respectively. The TNP-specific cell lines hy1.2, hy2.15, hy3.3 and hy5.19 contain approximately 100, 105, 106 and 99 chromosomes, respectively, and we suppose that fusion yielded hybrids that segregated better growing cells that had lost 7–14 chromosomes.

Evidence has been presented that X63-Ag8 cells preferentially fuse with the dividing cells of the spleen[2,6,7], which for the B-cell compartment consist mainly of Ig-secreting, plaque-forming cells. Sp2/0-Ag14 seems to display the same preference. In the population of spleen cells used for this fusion, approximately 0.4% of the cells made indirect (IgG) TNP-specific plaques and 0.004% made direct (IgM) TNP plaques. Assuming that 1% of the spleen cells are plaque forming cells[8] one would anticipate that about 40% of the hybridomas would make IgG with TNP specificity, as was observed. On the other hand, about 90% of the nonspecific plaque-forming cells of the spleen make IgM rather than IgG[8], so that one would anticipate that among the nonspecific hybridomas, 90% would also make IgM, a figure that is consistent with our observation that all four nonspecific hybridomas examined made IgM.

To obtain high-titre preparations of the TNP-specific Ig, 10^6 TNP-specific hybridoma cells were injected intraperitoneally into BALB/c mice. All caused tumours and yielded ascites fluid with titres generally about 100 times higher than for the culture supernatants.

The efficiency of fusion with Sp2/0-Ag14 has been variable. In our early experiments, such as the fusion described above, we obtained fewer hybrids than we ordinarily obtain with X63-Ag8. However, in more recent experiments, fusions with Sp2/0-Ag14 have often been more efficient, in particular in fusions with lipopolysaccharide stimulated spleen cells or with large spleen cells (F. Melchers, Clark and G.K., unpublished observations). We consider that Sp2/0-Ag14 can yield as many hybrids as X63-Ag8.

Alternative methods exist for obtaining hybridomas that do not express the myeloma Ig. It has been possible to isolate re-clones that have lost the expression of the nonspecific heavy or light chains[2]. This problem has been reduced by fusing with NSI-Ag 4/1, a derivative of X63 that makes the κ but not the $\gamma 1$ chain of the myeloma[2]. We anticipate that Sp2/0-Ag14 will prove yet a better cell line for generating hybridomas making truly monoclonal antibodies.

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Activation of latent Epstein–Barr virus by antibody to human IgM

EPSTEIN–BARR VIRUS (EBV) is the causative agent of most infectious mononucleosis[1] and is also associated with two human tumours, Burkitt's lymphoma (BL)[2] and nasopharyngeal carcinoma[2]. Lymphoid cell lines of B-cell origin have been established from patients with these diseases, and from lymphocytes transformed *in vitro* by EBV[3]. In lymphoid cell lines the lytic viral cycle is usually repressed even though the cells carry multiple copies of the EBV genome. These cells therefore provide a valuable model for the study of latent EBV infection. Although EBV is ubiquitous, its association with human malignant disease is to a large extent both ethnically and geographically restricted[2] and it is therefore of interest to understand the host factors involved in the regulation of the expression of the latent EBV genome. In infectious mononucleosis, EBV infection is associated not only with a specific anti-EBV antibody[2] and T-lymphocyte response[4] but also with an increase in nonspecific IgM production[5]. EBV-infected B lymphocytes produce polyclonal Ig (ref. 6) and the majority of established EBV genome-positive lymphoid cell lines possess surface IgM (ref. 7). We report here that treatment of several human lymphoid cell lines with antisera to human IgM activates the latent EBV genome to give a marked increase in EBV-specific early antigen (EA). Also, in some cell lines this treatment induces an increase in virus capsid antigen (VCA).

Treatment of BL-derived Raji cells with antiserum directed against human immunoglobulins increased the number of cells expressing EBV EA by up to 5,000-fold, to give 11% EA-positive cells determined by either direct or indirect immunofluorescence (Table 1), and confirmed by EA-specific complement fixation[8]. The fluorescence was specific for EA, as no positive cells were observed after staining with either control EBV-negative human serum, or with anti-VCA-positive EA-negative antiserum. EBV-specified antigens can be induced in lymphoid cell lines by halogenated pyrimidines[9,10], inhibitors of protein synthesis[11] or the tumour promotor 12-*O*-tetradecanoyl

Fig. 1 Induction of EA in cultures of Raji cells treated with various dilutions of antiserum. Cells were cultivated for 72 h with a particular dilution of antiserum in the presence of IUdR ($25 \mu g\ ml^{-1}$). The number of EA-positive cells was then determined on acetone-fixed smears by indirect immunofluorescence. None of the antisera had any effect on cell viability at the dilutions shown in the figure. ○, Sheep anti-total human immunoglobulins; ●, goat anti-human IgM; □, goat anti-human IgA; ■, goat anti-human IgG; △, goat anti-human IgD; ◇, goat anti-human IgE; ▲, IUdR alone ($25 \mu g\ ml^{-1}$).