The EMBO Journal vol.6 no.9 pp.2753–2758, 1987

Polymeric immunoglobulin M is secreted by transfectants of non-lymphoid cells in the absence of immunoglobulin J chain

Antonino Cattaneo and Michael S.Neuberger

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Communicated by C.Milstein

Plasmids were constructed in which expression of genes encoding the heavy and light chains of a hapten-specific IgM antibody is under control of a heat shock promoter. Glioma, phaeochromocytoma and other non-lymphoid cell lines transfected with the plasmids were able to process and secrete immunoglobulin following heat induction. The glioma transfectants were studied in detail and were shown to secrete polymeric IgM in a yield similar to that obtained with a plasmacytoma. However, the glioma IgM was not associated with J chain and was largely composed of pentamers and hexamers. Thus, neither J chain nor other lymphoid-specific proteins are required for assembly and secretion of polymeric IgM although the absence of J chain may encourage hexamer formation.

Key words: immunoglobulin M/J chain/secretion/glioma

Introduction

Antibodies are secreted by plasma cells, which represent the terminal stage of B cell differentiation. Whereas a plasma cell secretes large quantities of pentameric immunoglobulin, an IgM-expressing B cell contains monomeric IgM in its membrane but does not normally secrete antibody. The differentiation of a B cell into a plasma cell is not only accompanied by a large increase in the steady-state level of immunoglobulin mRNA, but there is also a shift from the production of mRNA for the membrane form of the heavy chain (μ_m) to the production of the secreted form (μ_s). However, whilst B cells do not on the whole secrete IgM, many B cells and B cell lymphomas are found to contain μ_s mRNA and intracellular μ_s polypeptide (Sibley *et al.*, 1980; Vassalli *et al.*, 1980; Sidman, 1981; Rubartelli *et al.*, 1983). Thus, whilst the difference in the production of secreted IgM by B cells and plasma cells can be in part ascribed to regulation at the level of RNA processing, it is clear that post-translational processes are also implicated.

Proteins involved in the assembly and secretion of the IgM pentamer could obviously play a role in this regulation. A lymphocyte-specific enzyme that may catalyse immunoglobulin assembly has been identified (Roth and Koshland, 1981) but a more popular candidate for fulfilling a regulatory function is immunoglobulin J chain (see Koshland, 1985 for a review). Immunoglobulin J chain is associated with secreted IgM and IgA (Halpern and Koshland, 1970; Mestecky *et al.*, 1971) and is synthesized by plasma cells [regardless of heavy chain class expressed (Kaji and Parkhouse, 1974 and 1975; Mosmann *et al.*, 1978)] but not by B cells (Roth *et al.*, 1979; Lamson and Koshland, 1984). The onset of J chain expression roughly coincides with the switch from membrane IgM to secreted IgM production (Roth *et al.*, 1979).

We were therefore interested in determining whether non-lymphoid cells could assemble and secrete IgM. Whereas IgM is secreted by transfected plasmacytoma cells (Neuberger, 1983; Ochi *et al.*, 1983; Neuberger *et al.*, 1984), the introduction of vectors driving expression of immunoglobulin cDNAs into yeast or *Escherichia coli* hosts has not resulted in effective antibody production — problems being encountered both with efficient assembly and with secretion (Cabilly *et al.*, 1983; Boss *et al.*, 1984; Wood *et al.*, 1985). It has, however, been found that IgG was secreted by *Xenopus* oocytes that had been injected with immunoglobulin mRNA (Colman *et al.*, 1982), although it is not

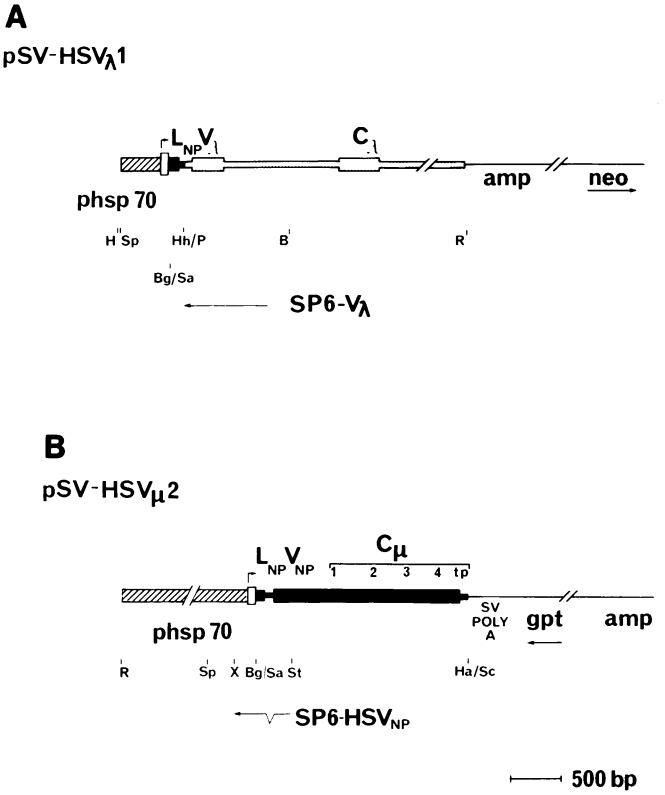

Fig. 1. Structure of plasmids. (**A**) Structure of pSV-HSVλ1. The thin line denotes the pSV2neo vector. Sequences of the immunoglobulin λ_1 light chain gene are stippled, the *Drosophila hsp70* promoter region (phsp70) is hatched, the leader exon derived from the heavy chain variable region (L_{NP}) is depicted by a filled box and the open box denotes a 60 nucleotide stretch of the 5′-untranslated region of *tk*. (**B**) Structure of pSV-HSVμ2. The various DNA segments are depicted as for pSV-HSVλ1 except that the vector (thin line) is pSV2gpt. The DNA encoding the μ constant region is derived from a cDNA encoding the Cμ1,2,3 and 4 domains as well as the secreted tailpiece (tp). The HS-Vμ2 transcription unit uses the SV40 polyadenylation site (SV POLY A) of pSV2gpt. The transcription start sites are indicated by an arrow and the probes used for ribonuclease protection assays (SP6-V_λ and SP6-HSV_{NP}) are also indicated; the SP6-HSV_{NP} probe lacks the L_{NP}-V_{NP} intron. Restriction sites are abbreviated: B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; Ha, *Hae*II; Hh, *Hha*I; P, *Pst*I; Sa, *Sau*3AI; Sc, *Sac*I; Sp, *Sph*I; St, *Stu*I; X, *Xho*I. Note that not all the sites for any one enzyme are shown — only those relevant to plasmid or probe construction are included.

Table I. Antibody yields from transfected cells

Cell line	Cell type	Concentration of IgM (μg/ml)
J[HSVμ2]	Plasmacytoma	0.5
C6[HSVμ2/HSVλ1]	Glioma	0.6
PC12[HSVμ2/HSVλ1]	Phaeochromocytoma	0.4
CHO[HSVμ2/HSVλ1]	Fibroblast	0.01
HeLa[HSVμ2/HSVλ1]	Epithelial	0.005
JW1/2/2[a]	Plasmacytoma	3.5

[a]This cell line secretes IgM constitutively; all other lines show heat-inducible antibody production.
The concentration of IgM was determined by radioimmunoassay on antigen-coated plates using purified JW1/2/2 IgM for calibration. Cells (3×10^6/ml initial concentration) were incubated at 37°C for 3 days with 1 h heat shocks (42.5°C) every 24 h.

possible to make a meaningful comparison of the efficiency of the secretion from injected oocytes to that from plasma cells. Here, we describe experiments to determine whether mammalian cell lines of non-lymphoid origin can assemble and secrete polymeric IgM and compare the efficiency of the process to that obtained using a plasmacytoma host.

Results

Inducible immunoglobulin genes

Plasmids were constructed in which transcription of the coding regions for the immunoglobulin heavy and light chains is under control of the promoter of the *Drosophila hsp70* heat-shock gene. The plasmid for heavy-chain expression, pSV-HSVμ2 (Figure 1B), includes the bulk of the cDNA for the immunoglobulin μ polypeptide with the *hsp70* promoter at the 5′ end and the SV40 polyadenylation signal at the 3′ end. The transcription unit contains a single intron (between the regions encoding the bulk of the leader peptide and V_HDJ_H) and is linked to a selective marker, *gpt*. The plasmid for light chain expression, pSV-HSVλ1 (Figure 1A), includes the expressed, genomic λ_1 gene of the mouse myeloma HOPC 2020 but with the promoter and leader exon replaced by the *hsp70* promoter/V_H-leader of plasmid pSV-HSVμ2. The HSVλ_1 light chain transcription unit is linked to the *neo* selective marker. Association of the polypeptides encoded by the two plasmids should yield an IgM,λ_1 antibody with specificity for the hapten 4-hydroxy-3-nitrophenacetyl (NP).

The activity of plasmid pSV-HSVμ2 in different environments was determined by introducing it into a number of cell lines; stable transfectants were screened for the expression of immunoglobulin gene mRNA and protein (Table I). As shown in Figure 2A, three different pools of HeLa cells transfected with pSV-HSVλ1 show inducible λ gene transcription; the fourth pool transcribes the gene constitutively. Similarly, pSV-HSVμ2 transfectants of the mouse plasmacytoma J558L show inducible transcription of the introduced heavy-chain gene (Figure 2B); the size of the fragment obtained in the RNase protection assays indicates that the transcripts are initiated by the *hsp70* promoter. It was confirmed by Northern blotting that the HSVμ2 and HSVλ1 transcripts were indeed of the expected size (data not shown).

Secretion of glycosylated IgM by non-lymphoid transfectants

Analysis of cells transfected with pSV-HSVλl revealed that most of these transfectants secreted λ_1 light chains into the culture supernatant. Clones of the transfected rat phaeochromocytoma line PC12 and of the rat glioma C6 were analysed for λ polypeptide synthesis. Whilst there was clonal variation in the amount of λ expressed (some *neo*$^+$ clones being negative for λ_1 expression), it was clear from immunoprecipitation experiments that heat induction of many of the C6 and PC12 transfectants resulted in the secretion of λ light chains (Figure 3A). Interestingly, the amount secreted by these transfectants is comparable to the amount of constitutive light chain secretion by the mouse plasmacytoma J558L (Figure 3A), despite the fact that J558L contains appreciably more λ_1 mRNA (not shown).

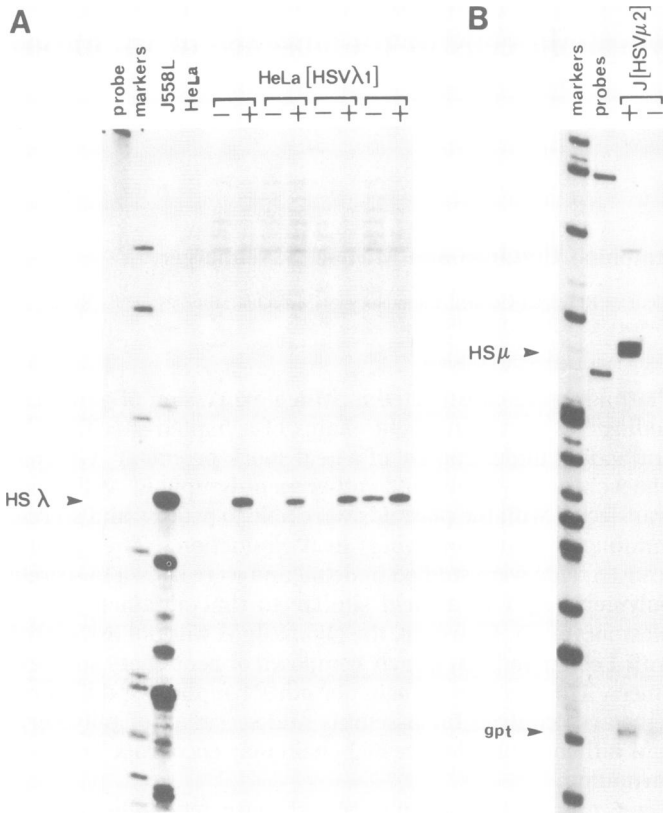

Fig. 2. Analysis of immunoglobulin mRNA in transfectants. (**A**) Assay of λ_1 mRNA in HeLa transfectants. Ribonuclease protection assays were carried out using the SP6-V_λ probe and cytoplasmic RNA from four pools of HeLa cells stably transfected with pSV-HSVλ1. RNA was prepared from cells before (−) and after (+) heat shock. RNA from the plasmacytoma J558L served as a control and an end-labelled *Hpa*II digest of pBR322 provided size markers. The protected fragment (HSλ) is the length of the entire V_λ exon. (**B**) Assay of HS-Vμ2 RNA in J558L transfectant J[HSVμ2]. The ribonuclease protection assay was carried out on induced (+) and uninduced (−) cells using a probe for the 5′-end of the HS-Vμ2 transcript as well as for *gpt* mRNA.

In order to test whether the phaeochromocytoma and glioma lines were able to assemble complete immunoglobulin, we analysed biosynthetically labelled proteins of PC12 and C6 cells that had been transfected with both pSV-HSVμ2 and pSV-HSVλl. The results (Figure 3B) show that heat shocking of both cell lines results in induction of the synthesis of μ polypeptide. The μ heavy chain is associated with λ_1 light chain and is glycosylated as judged by the effect of the glycosylation inhibitor tunicamycin (Figure 3B).

The C6 glioma transfectants were able to secrete the associated μ and λ polypeptides and the antibody secreted by these glioma transfectants was similar to that secreted by the plasmacytoma transfectant JW1/2/2 in that both antibodies bound the hapten NP (Figure 3D) and were recognized by anti-idiotypic antibodies

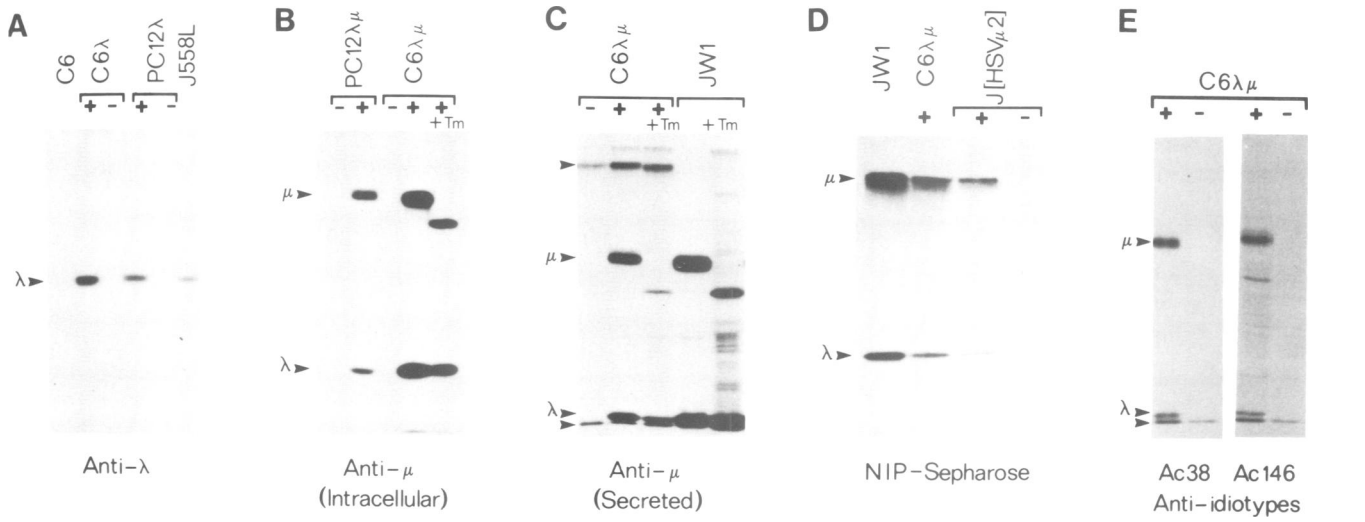

Fig. 3. Immunoglobulin secretion from transfected cells before (−) or after (+) heat-shock and in the absence or presence of tunicamycin (+Tm). (**A**) Light chain secretion by J558L and C6 and by pSV-HSVλ1 transfectants of C6 and PC12. Samples were immunoprecipitated from culture supernatants using anti-λ antiserum. (**B**) Intracellular immunoglobulin in PC12 and C6 clones that had been transfected with both heavy and light chain expression plasmids. After biosynthetic labelling, cytoplasmic samples were immunoprecipitated with anti-μ antiserum. (**C**) Secretion of IgM by C6[HSVλ1/HSVμ2] transfectant and by constitutive IgM-secreting J558L transfectant, JW1/2/2. After biosynthetic labelling, samples from the supernatant were precipitated with anti-μ antiserum. The high molecular weight band in this gel that is indicated by an arrow is also precipitated from untransfected C6 cells by anti-μ antibodies and reflects serological cross-reaction (A.C., unpublished observations). The other arrow indicates the dye-front. (**D**) Comparison of IgM secretion by JW1/2/2 (constitutive plasmacytoma expression), C6[HSVλ1/HSVμ2] (heat-inducible glioma transfectant) and J[HSVμ2] (which is a pSV-HSVμ2 transfectant of J558L and is therefore a heat-inducible IgM secreter). All samples were prepared following heat shock and analysed in parallel except that only half the JW1/2/2 sample was loaded on the gel and an incubation of J[HSVμ2] cells in the absence of heat-shock was performed for the right hand lane. IgM from culture supernatants was purified by adsorption onto NIPcap-Sepharose and eluted with I mM NIPcapOH. (**E**) Immunoprecipitation of glioma IgM with anti-idiotypic antibodies. Immunoglobulin in the supernatant of biosynthetically labelled C6[HSVμ2/HSVλ1] cells prior to (−) or following heat shock (+) was precipitated using monoclonal anti-idiotypic antibody Ac38 or Ac146 (Reth *et al.*, 1979).

(Figure 3E). Furthermore, the extent of glycosylation of the μ polypeptide secreted by the two cell types appears similar as judged by mobility in SDS/polyacrylamide gels.

To compare the efficiency of antibody secretion by a glioma transfectant to that by a plasmacytoma, we used the C6 double transfectant C6[HSVμ2/HSVλ1] and the J558L transfectant J[HSVμ2], which expresses an inducible μ gene from the transfected pSV-HSVμ2 plasmid and constitutively transcribes its endogenous λ_1 gene. In both transfectants, heat shocking leads to induction of μ synthesis in at least 80% of the cells as judged by cytoplasmic immunofluorescence. Following periodic heat pulses, the concentration of hapten-specific IgM in the culture supernatants was estimated by radioimmunoassay. The results (Table I) confirm that the level of inducible secretion is similar in the glioma and plasmacytoma transfectants — a conclusion which is in keeping with the results of biosynthetic labelling (Figure 3D). However, it is worth noting that the antibody yields obtained from the HeLa and CHO cell transfectants were considerably lower (Table I).

IgM secreted by glioma transfectants is polymeric and does not contain J chain

Secreted IgM is produced by plasma cells as a pentameric $(\mu_2L_2)_5$ structure (Miller and Metzger, 1965a,b; Parkhouse *et al.*, 1970) that is linked by disulphide bonds and contains a single, covalently-linked molecule of J chain polypeptide per IgM pentamer (Mestecky *et al.*, 1971). As it has been speculated (see Koshland, 1985) that J chain is necessary for IgM secretion and as J chain expression appears to be restricted to mature cells of the B lymphocyte lineage (Roth *et al.*, 1979), it was important to ascertain whether the IgM secreted by the glioma transfectants did, in fact, contain immunoglobulin J chain.

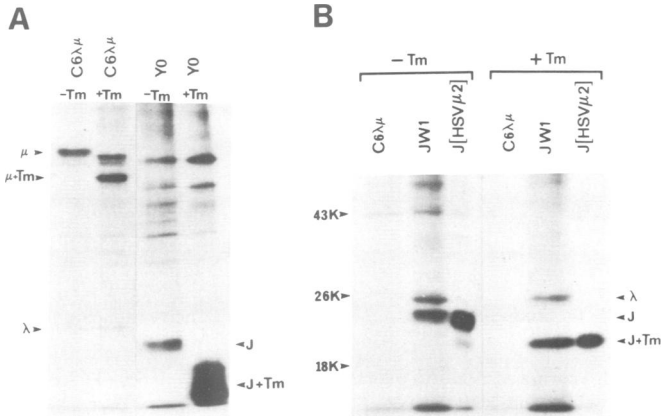

Fig. 4. Analysis for the presence of J chain polypeptide. A rabbit antiserum against J chain was used to precipitate J chain from biosynthetically labelled cell extracts that had been made in the presence or absence of tunicamycin (Tm) and that had been pre-cleared with anti-light and anti-heavy chain antisera and protein A sepharose. Nevertheless, it will be seen that precipitation with the anti-J chain antiserum does bring down some residual immunoglobulin polypeptides. (**A**) Rat YO myeloma cells and cells of the C6[HSVμ2/HSVλ1] transfectant that had been heat-induced 5 h previously were incubated in labelling medium for 5 h. (**B**) JW1/2/2 cells and cells of the J[HSVμ2] and C6[HSVμ2/HSVλ1] transfectants that had been heat-induced 7 h previously were incubated in labelling medium for 1 h.

To determine whether there was any intracellular J chain in C6 cells, immunoprecipitation was carried with an anti-J chain antiserum using cytoplasmic extracts of biosynthetically labelled cells. The results (Figure 4A and 4B) show that J chain can be detected in extracts of the plasmacytoma transfectants but not in the C6 glioma sample. Various different labelling times were

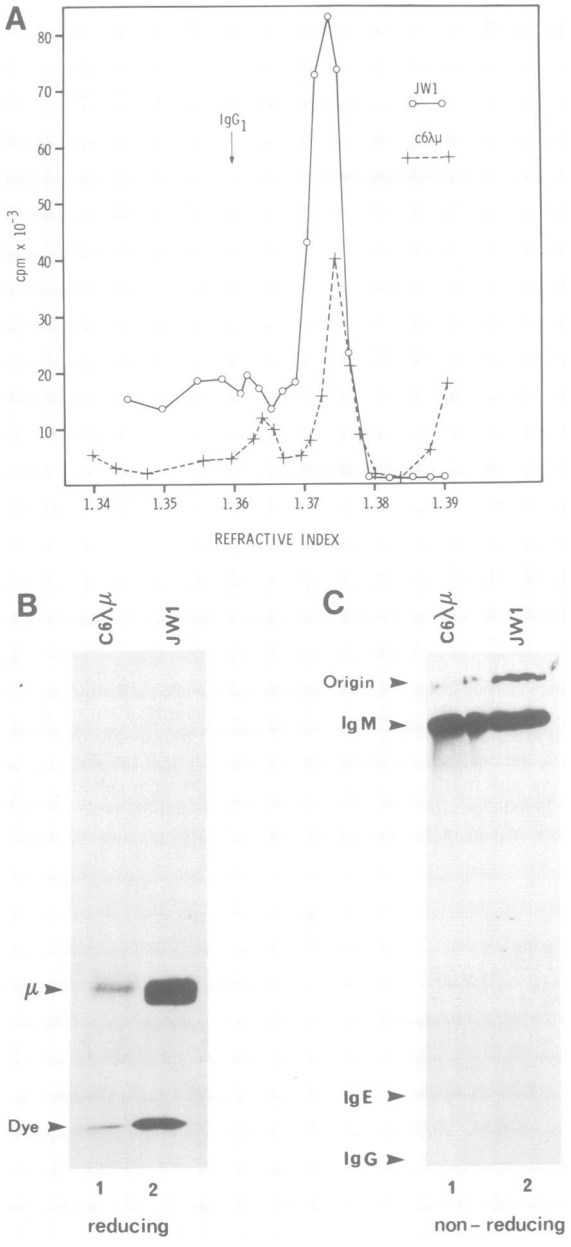

Fig. 5. IgM secreted from C6 glioma transfectant is polymeric. (**A**) Analysis by sucrose gradient centrifugation. Supernatants (1 ml) from biosynthetically labelled C6[HSVμ2/HSVλ1] (+---+), plasmacytoma IgM-secreting transfectant JW1/2/2 (o---o) or IgG1-secreting hybridoma P8.86.9 [gift of Thereza Imanishi] were loaded onto a gradient (15 ml) of 10–40% sucrose in PBS and subjected to centrifugation in a Beckman SW40 rotor at 4°C for 18 h. Samples were collected, the refractive indices determined and anti-NP antibody measured by radioimmunoassay. The supernatants from the C6 and J558L transfectants were analysed in parallel. (**B**,**C**) Analysis by polyacrylamide gel electrophoresis. Anti-NP antibody in the supernatants of biosynthetically labelled JW1/2/2 (labelled as JW1) or of heat-induced C6[HSVμ2/HSVλ1] was purified on hapten sorbents and analysed either (left panel, **B**) after reduction on a 7.5% SDS/polyacrylamide gel or (right panel, **C**) unreduced on a 4% SDS/polyacrylamide gel made using N,N′-diallyltartardiamide to cross-link. The positions of the origin, IgM, IgE, IgG and μ markers are indicated.

used as it has been reported (Mosmann *et al.*, 1978) that the half-life of J chain differs considerably in different cell lines. The detection of J chain polypeptide in the YO rat myeloma demonstrates that the anti-mouse J chain antiserum that was used will indeed precipitate rat J chain — a point that needed confirming as C6 is a glioma of rat origin. Not only was no J chain polypeptide detected in C6 cells, but it also appeared that these cells did not contain J chain mRNA as judged by primer extension assays (data not shown). Furthermore, analysis of IgM secreted by the plasmacytoma and C6 transfectants in alkaline/urea polyacrylamide gels after reduction and alkylation revealed J chain to be present only in the plasmacytoma sample (data not shown).

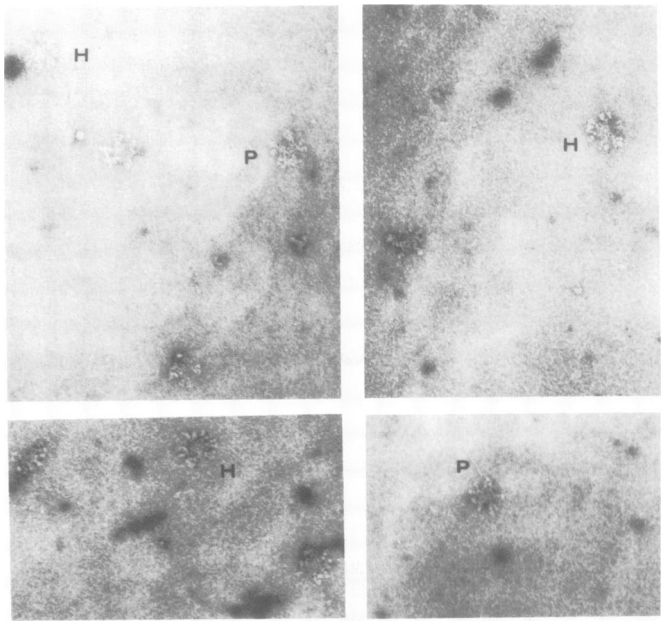

Fig. 6. Electron micrographs of negatively stained IgM molecules purified on hapten sorbents from the supernatant of heat-induced C6[HSVμ2/HSVλ1] transfectants. Different fields are shown in which both hexamers (H) and pentamers (P) are visible.

The sizes of the IgM secreted by the plasmacytoma and glioma transfectants were compared using both sucrose gradient centrifugation (Figure 5A) and electrophoresis through non-reducing SDS/polyacrylamide gels (Figure 5B). In both analyses, the IgM secreted by the glioma transfectant behaved exactly like the IgM secreted by the plasmacytoma and revealed itself to be a high molecular weight, covalently-associated aggregate. The resolution of the centrifugation and gel electrophoresis experiments was not, however, sufficient to demonstrate that the polymeric IgM secreted by the glioma transfectants was of exactly the same size as that produced by the plasmacytoma cells. We therefore resorted to an analysis of negatively stained protein samples in the electron microscope (Figure 6). Comparison of the IgM samples prepared from plasmacytoma and glioma transfectants shows that whereas the IgM secreted by the plasmacytoma is almost exclusively pentameric, the sample prepared from the glioma transfectants is found to contain both cyclic hexamers and cyclic pentamers in approximately equal amounts. The sample prepared from C6 cells also contains some molecules which, at first sight, appear to be incompletely assembled structures. However, the same structures are seen in the plasmacytoma IgM sample and are most probably hexamers or pentamers that are not lying flat on the grid (see Feinstein and Munn, 1969). Analysis of IgM secreted from the heat-inducible plasmacytoma transfectant J[HSVμ2] revealed it to be exclusively pentameric, indicating that the presence of hexamers in the glioma sample is unlikely to be an artefact of heat shocking.

Finally, it is worth noting that pulse-chase experiments performed using biosynthetic labelling (not shown) revealed that the polymerization of the IgM molecules occurred prior to their secretion from the glioma or plasmacytoma cell.

Discussion

Several different non-lymphoid cell lines were transfected with immunoglobulin heavy and light chain genes whose expression was under control of a heat-shock promoter. Transfectants were obtained that showed inducible expression, demonstrating that non-lymphoid cells are capable of assembling, processing and secreting immunoglobulin. There was considerable variation in the yield of immunoglobulin obtained using different cell types as host. Whereas HeLa and CHO cells gave relatively poor yields, the amounts of antibody secreted by glioma and phaeochromocytoma transfectants were comparable to those obtained using a plasmacytoma host. The greater efficacy of C6 glioma or PC12 as opposed to CHO or HeLa transfectants in antibody secretion does not appear to be due to a difference in the induction of immunoglobulin mRNA synthesis but rather may reflect the fact that the glioma and phaeochromocytoma are secretory cell types and may therefore be better equipped for the production of secreted immunoglobulin.

The IgM secreted by the C6 transfectants is polymeric, notwithstanding the absence of immunoglobulin J chain. This was somewhat unexpected as it has been proposed that J chain is required for the assembly and secretion of IgM (Koshland, 1985). However, assembly of polymeric IgM in the absence of J chain has previously been shown to occur both *in vitro* (Kownatzki, 1973; Eskeland, 1974 and 1977) and *in vivo* (Stott, 1976) although in some cases the high molecular weight products have been shown to be non-covalently bonded (Wilde and Koshland, 1978). In light of the results presented here, it is worth considering what the role of J chain in plasma cells might be. It could be involved in catalysing the rate of IgM assembly — as has been suggested from *in vitro* experiments (A.Feinstein, personal communication) — or it could be involved in stabilizing the pentamers. Indeed, the IgM secreted by the glioma transfectants is part pentameric, part hexameric and it is conceivable that the presence of hexamers is a direct consequence of the absence of J chain. However, in this context, it is worth noting that whereas serum IgM — like the IgM secreted by the J558L transfectants — is pentameric, the IgM secreted by another mouse plasmacytoma (MOPC 104E) has been found to contain a very small proportion of hexamers, although J chain is expressed in this plasmacytoma (Parkhouse *et al.*, 1970). It is interesting that the IgM antibody of *Xenopus* is exclusively hexameric (Metzger, 1970; Parkhouse *et al.*, 1970); it is not clear whether *Xenopus* IgM contains J chain although a polypeptide similar to it has been described in amphibia (Weinheimer *et al.*, 1971).

Therefore, in summary, it is clear that J chain is not required for the production of polymeric IgM in good yield from the glioma transfectants. It is, however, possible that the IgM secreted by these transfectants is deficient in some functional aspect. Indeed, evidence has been published that indicates a requirement for J chain in the selective transport of polymeric human IgM through secretory cells into exocrine fluids (Brandtzaeg and Prydz, 1984). The activity of the glioma IgM in epithelial transport and complement activation will be tested. Alternatively, it is possible that J chain may be involved in catalysing the production of IgM pentamers, as opposed to a pentamer/hexamer mixture. This can be tested by introducing an inducible J chain gene into both B cell lymphomas and into the glioma transfectants.

Both PC12 and C6 cells are derived from cell types present in the nervous system. The fact that transfectants of both cell types are able to synthesize and secrete immunoglobulin means that it should be possible to engineer the production of specific antibodies in the central nervous system of transgenic organisms in order to perturb or modulate the activity of selected neuronal pathways.

Materials and methods

Plasmid construction

Plasmid pSV-HSVμ2 includes the HS-Vμ2 transcription unit cloned into a derivative of pSV2gpt (Mulligan and Berg, 1981) in which the *Bam*HI site has been converted to a *Sac*I site by use of linkers. The HS-Vμ2 transcription unit was assembled in three parts. The promoter/transcription start region was obtained from plasmid pF1 of Pelham (1982) as an *Eco*R−*Bgl*II fragment and contains the *Drosophila hsp70* promoter from an *Eco*RI site at the 5′ end to the *Sal*I site immediately 3′ of the TATA where it is fused to the 60 nucleotide *Hae*III−*Bgl*II fragment of herpes simplex virus *tk* which includes the RNA cap site. The DNA encoding the leader region of the heavy chain polypeptide (L_{NP}) was obtained from mouse V_H gene V-47 (Neuberger, 1983) and extends from the *Sau*3AI site at the transcription start through the leader-V_{NP} intron to a *Pst*I site at codon +5 of V_{NP} (Neuberger, 1983). The rest of the heavy chain coding region is derived from a cDNA clone for the heavy chain of an NP-specific antibody [pABμ11 (Bothwell *et al.*, 1981)] and extends from the *Pst*I site at codon +5 to a *Hae*II site in the Cμ 3′-untranslated region. After final assembly of pSV-HSVμ2, the *Hae*II site at the 3′ end of Cμ is brought adjacent to the *Sac*I site in the derivatized pSV2gpt vector. Plasmid pSV-HSVμ2 is a cDNA derivative of pSV-HSVμl (Mason, 1987).

Plasmid pSV-HSVλl is based on a derivative of pSV2neo (Southern and Berg, 1982) in which the *Hin*dIII site has been destroyed by filling in and a new *Hin*dIII site created by linker insertion in the *Bam*HI site. The promoter/transcription start/leader region is the same as in pSV-HSVμ2 except (i) that the region 5′ of the promoter extends only as far as the *Sph*I site, and (ii) the L_{NP}-V_{NP} intron is truncated at an *Hha*I site. This *Hha*I site in the L_{NP}-V_{NP} intron is fused to a *Pst*I site in the L_λ-V_λ intron of the expressed λ_1 gene of the mouse plasmacytoma HOPC 2020 (Brack and Tonegawa, 1977), which provides the rest of the transcription unit.

The probe for mapping HS-Vμ2 transcripts was obtained by subcloning into pSP65 a fragment derived from an intronless derivative of pSV-HSVμ2 that extends from the *Stu*I site in V_{NP} back to the *Xho*I site in the *hsp70* promoter. The probe for light chain expression was obtained by subcloning the *Bam*HI−*Pst*I V_λ fragment of the HOPC 2020 light chain gene into plasmid pSP65.

Cell lines and transfection

Mouse plasmacytoma J558L (Oi *et al.*, 1983), its IgM-secreting transfectant JW1/2/2 (Neuberger *et al.*, 1984), rat myeloma YO (Galfré and Milstein, 1981), C6 rat glioma (Benda *et al.*, 1968) and chinese hamster ovary (CHO) and HeLa cells were grown in DMEM containing 10% FCS whereas PC12 cells (Greene and Tischler, 1976) were grown in RPMI containing 10% heat-inactivated horse serum and 5% FCS. The phenotype of the C6 glioma cells and its transfectants was confirmed by measuring receptors for nerve growth factor (NGF) (Cattaneo *et al.*, 1983), fluorescent staining with antibody MC 192 (Chandler *et al.*, 1984), immunoprecipitation of S100 protein (Labourdette and Mandel, 1978) and immunofluorescent staining for glial fibrillar acidic protein (Raju *et al.*, 1980). The PC12 cell transfectants were shown to respond to NGF. Transfection by spheroplast fusion was carried out as previously described (Neuberger, 1983) and electroporation (Neumann *et al.*, 1982) was performed by giving four 2 kV pulses to a suspension of 2.5×10^7 cells in 0.5 ml PBS containing 20 μg of supercoiled plasmid DNA. Transfection of J558L and C6 glioma was by spheroplast fusion, of PC12 cells by electroporation and of HeLa and CHO by calcium phosphate co-precipitation. For selection of *neo* transfectants, G418 was used at 0.5 mg/ml (PC12), 1 mg/ml (C6) or 2 mg/ml (other cells); *gpt*$^+$ colonies were selected as described by Mulligan and Berg (1981) for non-lymphoid cells and as previously described (Neuberger *et al.*, 1984) for J558L.

Analysis of RNA

Total cytoplasmic RNA was prepared as previously described (Neuberger, 1983). For heat induction, RNA was extracted 4 h after the cells had been subjected to a 2 h shock at 42.5°C. Ribonuclease protection assays were performed as described by Melton *et al.* (1984).

Analysis of protein

For biosynthetic labelling of immunoglobulin, cells (5×10^6 in 1 ml) were heat-shocked at 42.5°C for 2 h, washed and resuspended in medium containing 5%

dialysed FCS, one-tenth the normal concentration of methionine and 50 μCi/ml L-[^{35}S]methionine (1220 Ci/mmol; Amersham International). After a 14–16 h incubation at 37°C, cells were collected by centrifugation, washed and lysed in 0.2 ml PBS/0.5% Nonidet-P40/1 mM phenylmethylsulphonyl fluoride (PMSF). For analysis of J chain, the labelling period was shorter (see Figure 6 legend) and cells were lysed in 0.1 M Tris–HCl (pH 8.0)/0.1 M KCl/5 mM $MgCl_2$/0.5% NP40/1 mM PMSF. When required, tunicamycin (8 μg/ml) was included during the heat-shock and labelling periods.

Immunoprecipitations from culture supernatants or cytoplasmic extracts was carried out by incubation with purified rabbit anti-mouse μ antiserum (kind gift of R.Sitia) or rat monoclonal antibody SM1/45 (kind gift of Y.Argon and M.R.Clark), goat anti-mouse λ (Miles-Yeda), monoclonal anti-idiotype antibodies Ac38 and Ac146 [kind gift of M.Reth (Reth *et al.*, 1979)] or rabbit anti-mouse J chain antiserum (kind gift of R.M.E.Parkhouse) followed by precipitation with protein A-Sepharose (Pharmacia). After washing with 50 mM Tris–HCl (pH 7.6)/1 M NaCl/0.25% NP40, samples were dissolved in reducing SDS sample buffer and analysed by electrophoresis through SDS/polyacrylamide gels (Laemmli, 1970). Unreduced samples were analysed on 4% acrylamide/0.7% N,N′-diallyltartardiamide gels (Pearson *et al.*, 1977). Purification on hapten sorbents was carried out using NIP-caproate linked to Sepharose as previously described (Neuberger *et al.*, 1984).

For analysis in the electron microscope (for which we are greatly indebted to John Finch), antibody that had been freshly affinity purified on hapten columns was concentrated to 1 mg/ml, layered onto carbon films and viewed by negative contrast following staining with uranyl acetate.

Acknowledgements

A.C. thanks David Secher for his constant support throughout this work and EMBO for a long-term fellowship. We also thank Cesar Milstein for encouragement and valuable advice, Roberto Sitia and Arnold Feinstein for helpful discussions and John Finch for vital assistance with electron microscopy. We are very grateful to Drs Berg, Bothwell, Hozumi, Mason, Parkhouse, Reth, Sitia and Tonegawa for DNA clones or antisera.

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Received on May 18, 1987

Note added in proof

The secretion of low levels of functional IgG from transfected COS and CHO cells has been recently reported [Weidle,U.H., Borgya,A., Mattes,R., Lenz,H. and Buckel,P. (1987) *Gene*, **51**, 21–29].