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Deletion of Cμ genes in mouse B lymphocytes upon stimulation with LPS

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Mouse B lymphocytes can be activated polyclonally by bacterial lipopolysaccharide (LPS) to differentiate into plasmablasts. Within several days many cells perform immunoglobulin (Ig) class switching *in vitro*. We have purified LPS blasts expressing IgM or only IgG3 on the cell surface and analysed the DNA of these cells by Southern hybridisation blotting to detect rearrangement or deletion of C_H genes. Quantitative evaluation of the Southern blots suggests that populations of surface IgG3$^+$ (sIgG3$^+$) cells from 6-day and sIgM$^+$ cells from 8-day-old cultures contain only about half as many Cμ genes as spleen cells. Cμ deletion is nearly complete in populations of sIgG3$^+$ cells from 9-day-old cultures. Therefore, upon stimulation with LPS, within a few days Cμ is deleted in most sIgG3$^+$ cells from both chromosomes.

Key words: LPS blast cells/Cμ deletion/fluorescence-activated cell sorting/isotype switch

Introduction

Lymphocytes of the mouse B-cell lineage undergo rearrangements of their immunoglobulin (Ig) genes at several stages of differentiation. The genes coding for V-, D- and J sequences of the prospective Ig heavy chains are joined to form a contiguous VDJ-gene (Early *et al.*, 1980; Sakano *et al.*, 1980). This gene is first expressed together with the Cμ gene that encodes the IgM heavy chain constant region (Melchers *et al.*, 1975; Cooper *et al.*, 1976; Raff, 1976). The B cell can switch from the expression of Cμ to the expression of other constant region (C_H) genes located downstream of Cμ on the chromosome. In plasmacytoma and hybridoma cells expressing isotypes other than IgM, the Cμ gene and all C_H genes located between the VDJ and the C_H gene that is expressed are deleted from the active IgH locus and often also from the inactive one (Rabbitts *et al.*, 1980; Cory and Adams, 1980; Davies *et al.*, 1980a, 1980b; Kataoka *et al.*, 1980; Coleclough *et al.*, 1980; Maki *et al.*, 1980; Cory *et al.*, 1980, 1981; Yaoita and Honjo, 1980; Hurwitz *et al.*, 1980). One question left open by the investigation of these transformed cell lines was whether the deletions were a prerequisite or a consequence of the isotype switch especially since these cell lines are likely to be derived from Ig-secreting plasma cells and thus represent a late B cell differentiation stage. The time span between the switching event and investigation of the IgH locus is not known for any of these cells. Furthermore, in small B cells expressing IgE and IgM on the surface, Cμ is probably not deleted from the active chromosome and it has therefore been postulated that the deletion is correlated to secretion of Ig rather than to switching of the isotype (Yaoita *et al.*, 1982).

Here we ask whether Cμ genes are deleted in B cells polyclonally activated to differentiate into plasmablasts by bacterial lipopolysaccharide (LPS). Upon stimulation, Ig class switching occurs in many clones (Kearney *et al.*,1976, 1978; Pernis *et al.*, 1976; Anderson *et al.*, 1978). Hurwitz and Cebra (1982) have shown that Cμ is rearranged in LPS blasts expressing low amounts of cytoplasmic IgM (cIgM). We used panning on anti-isotype antibody-coated Petri dishes and fluorescence-activated cell sorting with fluorochrome-conjugated isotype-specific antibodies to isolate LPS blasts expressing either IgM or only IgG3 on the cell surface. The DNA of such cells was then analysed by the Southern hybridisation technique (Southern, 1975; Wahl *et al.*, 1980).

Results

Isolation of LPS blast subpopulations

The experimental protocol for LPS stimulation and purification of blast cell subpopulations is given in Figure 1. Culture conditions were adjusted to give high proliferation rates as measured by tritiated thymidine incorporation (data not shown). Because of massive cell death after day 6, we had to purify live cells by centrifugation on Ficoll-isopaque before selection of blast cell subpopulations. This resulted in viable populations even on day 11 of culture (>90% viability). The selection of cells expressing surface IgG3 but not IgM is complicated by the fact that ~50% of the sIgG3$^+$ cells also stain

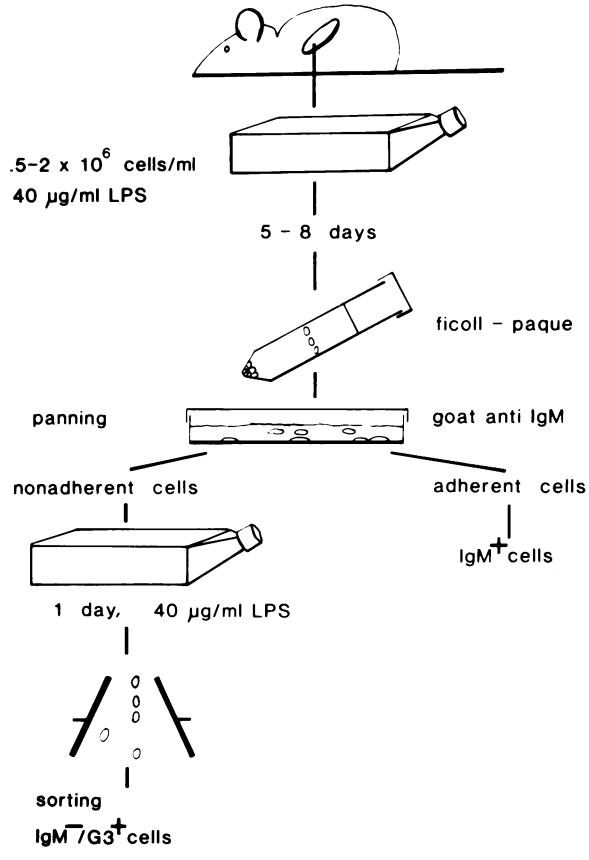

Fig. 1. Cell purification protocol. Details of the cell culture and purification procedures are given in Materials and methods.

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Table I. Frequencies of IgG3 expressing LPS blasts

(1)	sIgM	cIgM	sIgG3	cIgG3	sIgG3 + sIgM	sIgG3 + cIgM	sIgG3 + cIgG3	cIgG3 + sIgM	+sIgM + cIgG3
Day 0[a]	39	<1[b]	<1	–[c]	–	–	–	–	–
Day 6	34	31	15	10	57	2.6	24	50	14
Day 9	53	44	23	11	38	<1	27	43	7.4

(2)	sIgM	sIgG3	sIgG3 + sIgM	Dead or negative
Day 8	60	12	58	–
Day 9, IgM^-	<4	10	<5	–
Sorted cells	<1	>95	<1	<3

[a]The day of starting LPS culture was counted as day 0.
[b]Frequencies of <10% are given as approximate values if <300 cells were counted.
[c]These frequencies were not determined.
Surface (s) and cytoplasmic (c) Ig could be distinguished by the staining pattern. Frequencies are given in %.
(1) Frequencies of cells expressing IgM or IgG3 in a typical LPS mass culture maintained as described in Figure 1. Each number is based on >100 cells counted. Data obtained from other cultures were comparable although not identical. Double producers are given as the fraction of cells expressing the first type that also express the second type.
(2) Frequencies of $IgG3^+$ and/or IgM^+ cells through the process of selection. The purified cells were used for the restriction analysis shown in Figure 6, track 3.

for sIgM when analysed by fluorescence microscopy (Table I). We separated cells positive and negative for sIgM by panning the LPS blasts on goat anti-IgM-coated Petri dishes 1 day prior to cell sorting. The adherent cells were >90% IgM^+. They were collected and their DNA was subjected to Southern hybridisation analysis. The non-adherent cells were re-stimulated with LPS for 1 day and then stained for sIgM with Texas red-conjugated goat anti-IgM and for sIgG3 with fluorescein-conjugated goat anti-IgG3 antibodies. The isotype-specific antibodies were affinity purified and conjugated as previously described (Kearney *et al.*, 1976; Kearney and Lawton, 1975). Staining of IgG3 was specific because the goat anti-IgG3 antibody bound only to IgG3 antibodies in radioactive binding assays and the fluorescein-conjugated anti-IgG3 derived from it stained only $IgG3^+$ cells out of a panel of hybridoma cells (data not shown) and <1% of spleen cells (Table I). Between 10 and 30% of the LPS blasts were stained on days 6 and 9 of culture (Table I). It is unlikely that a major proportion of these $sIgG3^+$ cells absorbed the IgG3 passively rather than producing it themselves, because <2.6% of the $sIgG3^+$ cells co-stained for cIgM although in the whole population of LPS blasts 30–45% of the cells express cIgM (Table I). Furthermore, we could deplete the population of cells co-staining for sIgG3 and sIgM by panning on anti-IgM antibodies. Less than 5% of the non-adherent $sIgG3^+$ cells also stained for sIgM (Table I). $sIgG3^+$ cells were further purified by fluorescence-activated cell sorting.

For cell sorting we used four parameters (Figure 2): forward light scatter, propidium iodide staining and staining with Texas red-conjugated goat anti-IgM and fluorescein-conjugated goat anti-IgG3 antibodies.

Light scatter gating was used to exclude dead cells and cell clumps (Figure 2A) (Loken and Herzenberg, 1975). We also excluded dead cells by red fluorescence because, in contrast to live cells, they stained brightly with propidium iodide (Yeh *et al.*, 1981). The red fluorescence staining achieved with Texas red-anti-IgM is far less prominent than propidium iodide staining and positive cells are distributed over the display of the pulse height analyser. We deflected cells staining only with fluorescein-anti-IgG3 (see sorting windows in Figure 2C). An aliquot of the deflected cells was analysed by fluorescence microscopy to reveal the degree of purification. Usually, >95% of the sorted cells were $sIgG3^+/sIgM^-$ and alive. $1-3 \times 10^6$ $sIgG3^+$ cells were obtained from sorting of up to 6×10^7 scatter-positive LPS blasts per experiment.

Restriction endonuclease analysis

The DNA of the cells was subjected to restriction endonuclease analysis by the Southern hybridisation technique (Figure 3). The DNA probes used were specific for Cμ (Bothwell *et al.*, 1981) and the switch sequence located in front of the Cγ1 gene (sγ1) (Shimizu *et al.*, 1982). As restriction enzymes we used *Eco*RI and *Hind*III. *Eco*RI fragments of 12 kb (IgH^a) and 13.5 kb (IgH^b) contain the Cμ gene and its switch region (Marcu *et al.*, 1980). Digestion with *Eco*RI should therefore demonstrate gross rearrangements of Cμ by either disappearance of the germline bands in case of heterogeneous rearrangements or appearance of new bands if the same rearrangement occurred in a major fraction of the cell population. Digestion of mouse liver DNA with *Hind*III results in two fragments that carry the 3′ and 5′ halves of Cμ. The absence of the small (1.3 kb) 3′ Cμ fragment would amost certainly indicate the deletion of Cμ because it contains not much more than the Cμ3 and Cμ4 exons. Both *Hind*III and *Eco*RI digestions result in large germline sγ1 fragments differing in size for the a and b haplotypes (Shimizu *et al.*, 1982).

The digested DNA was size separated on agarose gels and transferred to nitrocellulose filters. These filters were first hybridised to the Cμ probe and subsequently, without intermediate washing, to the sγ1 probe. We could therefore use the intensity of the sγ1 bands to evaluate the amount of DNA on individual tracks and relate the amount of Cμ, i.e., the intensity of the Cμ bands, to it. We are aware that we might perhaps underestimate the amount of DNA and the extent of Cμ deletion if sγ1 sequences were deleted in certain popula-

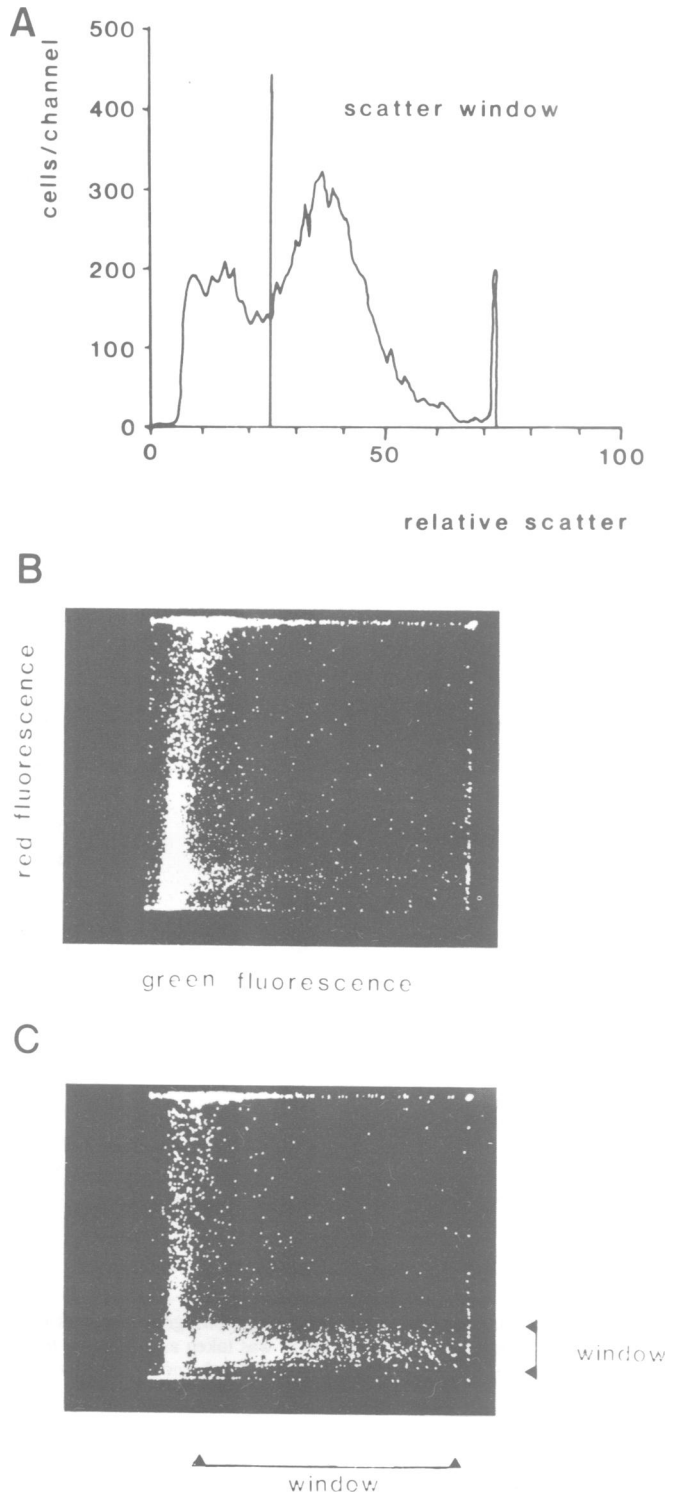

Fig. 2. Fluorescence-activated cell sorting. **(A)** Scatter profile of a 9-day-old LPS culture treated as described in Figure 1. Bright cells are viewed alive and are in the scatter window. The red and green fluorescence of these cells as displayed on a X-Y oscilloscope is shown in **(B)** which is a plot of red *versus* green fluorescence of individual scatter positive cells stained with goat anti-IgM-Texas red and goat anti-IgG3-fluorescein and analysed in the presence of propidium iodide. Fluorescence is increasing from left to right and bottom to top. Each dot marks the relative fluorescence of one cell. Cells on the top, although-scatter positive, were dead as they took up propidium iodide and were brightly red fluorescent. **(C)** Same plot as in **(B)** with superimposed fluorescence windows. Cells staining only with fluorescein-anti-IgG3 and thus showing low red and high green fluorescence appear within the windows. These cells were deflected.

tions. Our experiments thus give minimal estimates of Cμ deletion.

The results of the restriction analysis are shown in Table II and Figures 4, 5 and 6. While Figures 4–6 show autoradiographs of the Southern blots, in Table II we have summarised our attempts to obtain quantitative estimates of the band intensities on these films.

In Figure 4 (filter 194) the *Hind*III-digested DNA of sIgG3$^+$ from 6-day and sIgM$^+$ LPS blasts from 8-day-old cultures is compared with the DNA of (C57BL/6 x BALB/c)F_1 spleen cells and C57BL/6 liver cells. It is not obvious from the figure but clear from the photometric scanning (Table II, filter 194) that both LPS blast populations have about half as many germline Cμ genes relative to sγ1 sequences as do spleen or liver cells. The reduction of Cμ band intensity is also seen in *Eco*RI-digested DNA of sIgM$^+$ blast cells from a different experiment (Table II, filter 188). For sIgG3$^+$ cells from 6-day-old cultures, the 2-fold reduction in Cμ gene content when compared with spleen cells is also confirmed by a second preparation (Table II, filter 185). On day 9 of LPS stimulation, hardly any germline *Hind*III Cμ bands can be detected in sIgG3$^+$ blast populations while the germline sγ1 fragments are as frequent as in control DNA (Figure 5, filter 191). This filter is not included in Table II because only the peaks of the sγ1 hybridisation are in the linear dose-response range of the film while those of the Cμ bands are not. However, the reduction in signal intensity is quite obvious and the result is confirmed by the analysis of *Eco*RI-digested DNA from two independently isolated populations of day 9 sIgG3$^+$ cells (Figure 6, filter 188). One preparation of sIgG3$^+$ blasts shows only faint germline Cμ bands (lane 3) while the other shows no apparent Cμ bands (lane 4). All four lanes have comparable amounts of germline sγ1 fragments (Table II, filter 188). The quantitative estimate is ~16% and 12% of Cμ genes in these sIgG3$^+$ LPS blast populations when compared with liver cells. This corresponds to the lane background in the case of Figure 6, lane 4.

Discussion

From the *Hind*III and *Eco*RI analysis of sIgG3$^+$ blast cells of 9-day-old cultures (Figures 5 and 6) we conclude that the Cμ gene is deleted from both chromosomes in most of these cells. If, at most, 16% of the Cμ genes are retained in the population (Figure 6, lane 3, Table II) then at least 70% of day 9 sIgG3$^+$ cells have deleted Cμ from both chromosomes. This is not too different from the situation in anti-phosphorylcholine antibody secreting hybridoma cell lines where in 30% (two out of six) of the IgG3$^+$ or IgG1$^+$ lines, Cμ is clearly deleted from both chromosomes (Hurwitz *et al.*, 1980).

Comparing sIgG3$^+$ LPS blasts from 6- and 9-day-old cultures it would appear that deletion of Cμ takes place during LPS stimulation. While about half of the Cμ genes in sIgG3$^+$ blast populations are probably deleted until day 6, nearly all Cμ genes are deleted until day 9 of LPS culture. Populations of both days had been purified to the same degree. On both days the relative numbers of cells expressing sIgM, cIgM, sIgG3 or cIgG3 or various combinations of these are similar (Table I). We did not find a change in any of these parameters that was strikingly correlated with the Cμ deletions occurring between days 6 and 9 of culture. This is probably also true for Ig secretion since, although all sIgG3$^+$ cells are probably blast cells, only 25% of them stain for

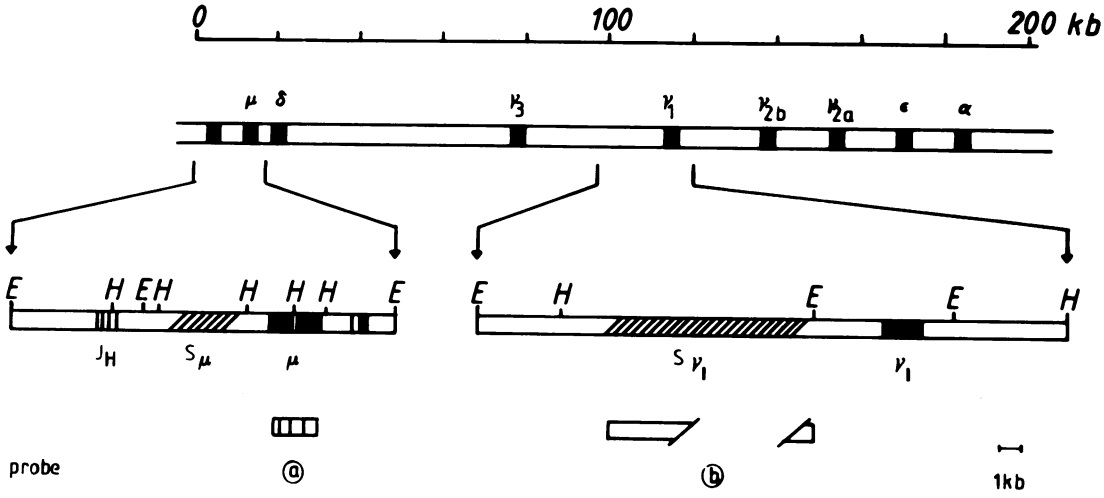

Fig. 3. Restriction enzymes and molecular probes. The IgH locus of BALB/c mice is according to Shimizu *et al.* (1982). Exons are indicated by black boxes, switch region sequences are shaded. (H) *Hind*III, (E) *Eco*RI. Differences between the 'a' (BALB/c) and 'b' (C57BL/6) haplotypes have been described for the length of sμ and sγ1 (Shimizu *et al.*, 1982; Marcu *et al.*, 1980). They are not shown in the figure. Probe (a) is specific for Cμ and probe (b) highly specific for sγ1.

Table II. Relative amount of Cμ genes in sIgM$^+$ and sIgG3$^+$ LPS blast cell populations

Filter	Enzyme	Mouse strain	Cell type	Age of culture	Rel. DNA content	Rel. Cμ content
		C57BL/6	Liver	–	31	100
194		F1[a]	Spleen	–	37	95
Figure 4	*Hind*III	F1	IgM$^+$[b]	8 days	69	54
		F1	IgG3$^+$	6 days	100	46
		C57BL/6	Liver	–	91	100
185	*Hind*III	C57BL/6	IgM$^+$ spleen[c]	–	91	80
		F1	IgG3$^+$	6 days	100	48
		C57BL/6	Liver	–	44	100
188		F1	IgM$^+$	8 days	100	63
Figure 6	*Eco*RI	F1	IgG3$^+$	9 days	91	<16
		F1	IgG3$^+$	9 days	79	<12

[a](C57BL/6 x BALB/c)F_1 mice.
[b]LPS blast cell subpopulations if not indicated otherwise.
[c]Cells were purified by cell sorting of spleen cells stained with fluorescein-conjugated goat anti-IgM.
Only peaks within the linear dose response range of the film were evaluated for this table except the Cμ peaks of sIgG3$^+$ blasts from 9-day-old cultures. Whenever two bands were derived from hybridisation to one probe the sum of both peaks was taken as one. Even in lanes with high background signals (Cμ in filter 185 and 188, lane 2) only the background of the film, not that of the lane, was subtracted. The height of the sγ1 peaks was taken as a measure for the amount of DNA per lane. The relative amounts of DNA are given in %. The relative Cμ content of a population is then given by the normalised ratio of Cμ and sγ1 peak heights.

cIgG3 as well on both days.

A third point that emerges from our analysis is that about half of the Cμ genes are probably deleted from sIgM$^+$ blasts of 8 day LPS cultures. This finding was unexpected since, according to the analysis of myeloma and hybridoma cells, deletion of Cμ appeared to be confined to cells expressing C_H genes other than Cμ. The result should therefore await confirmation by an independent method. However, if indeed in the day 8 sIgM$^+$ blast cell populations all cells do not have two Cμ genes this still could have a trivial explanation. We do not know how many cells of the sIgM$^+$ blast populations are transcribing a Cμ gene. One could imagine that ~50% of day 8 sIgM$^+$ blasts underwent isotype switching and have deleted Cμ from both chromosomes as have day 9 sIgG3$^+$ cells. These sIgM$^+$ cells would no longer transcribe Cμ but would express it due to 'stable' mRNA, persistent IgM molecules or passively absorbed IgM. This is in fact possible because quite a large fraction of sIgM$^+$ blasts (~20%) also expresses sIgG3$^+$ (Table I) and, assuming equal chances for IgG3 and some other IgG isotypes to be co-expressed with IgM on the cell surface (Coutinho and Forni, 1982), >50% of the sIgM$^+$ LPS blasts will express IgM and another Ig class around day 8 of culture. If in most of these cells Cμ were deleted from both chromosomes, Cμ would be deleted from the total sIgM$^+$ population to ~50%.

If, on the other hand, most of the day 8 sIgM$^+$ blast cells that we analysed are transcribing a Cμ gene but indeed have deleted Cμ from one chromosome, we would have to suggest

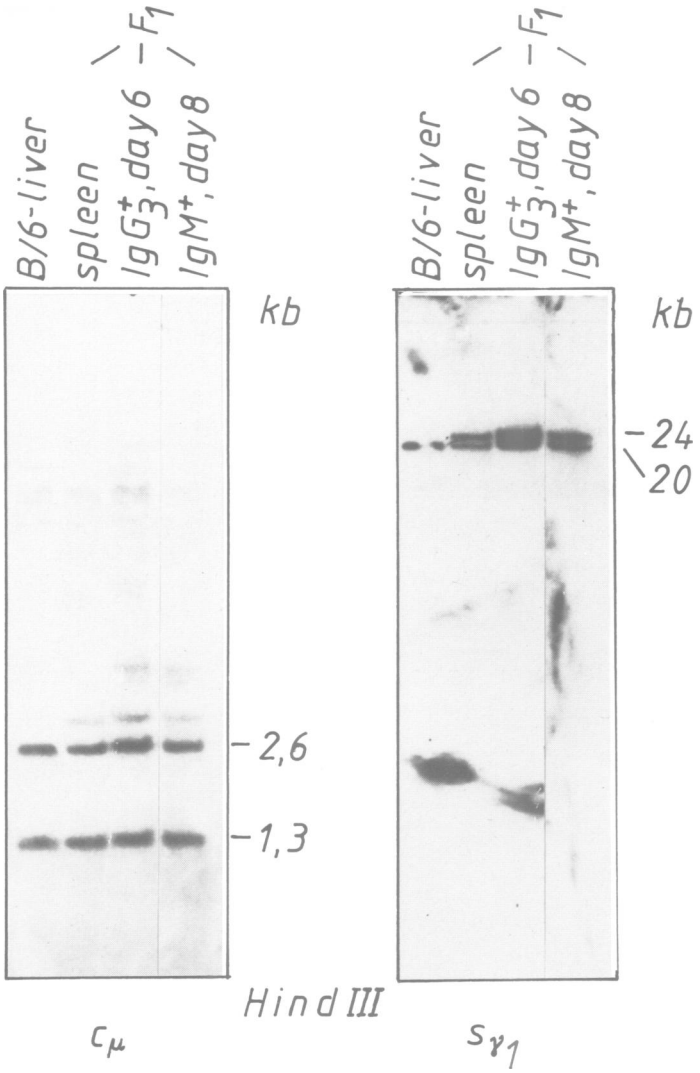

Fig. 4. DNA of C57BL/6 liver cells, (C57BL/6 x BALB/c)F1 (F1) spleen cells, IgM^+ LPS blasts from an 8-day-old culture and $IgG3^+$ cells from a 6-day-old culture was cut with *Hind*III and analysed by Southern blotting to the Cμ-specific probe and then without washing to the sγ1 probe. The two bands of sγ1 hybridisations correspond to the a and b alleles of the sγ1 fragments while the two bands of Cμ hybridisations correspond to the 3′ and 5′ ends of the Cμ gene.

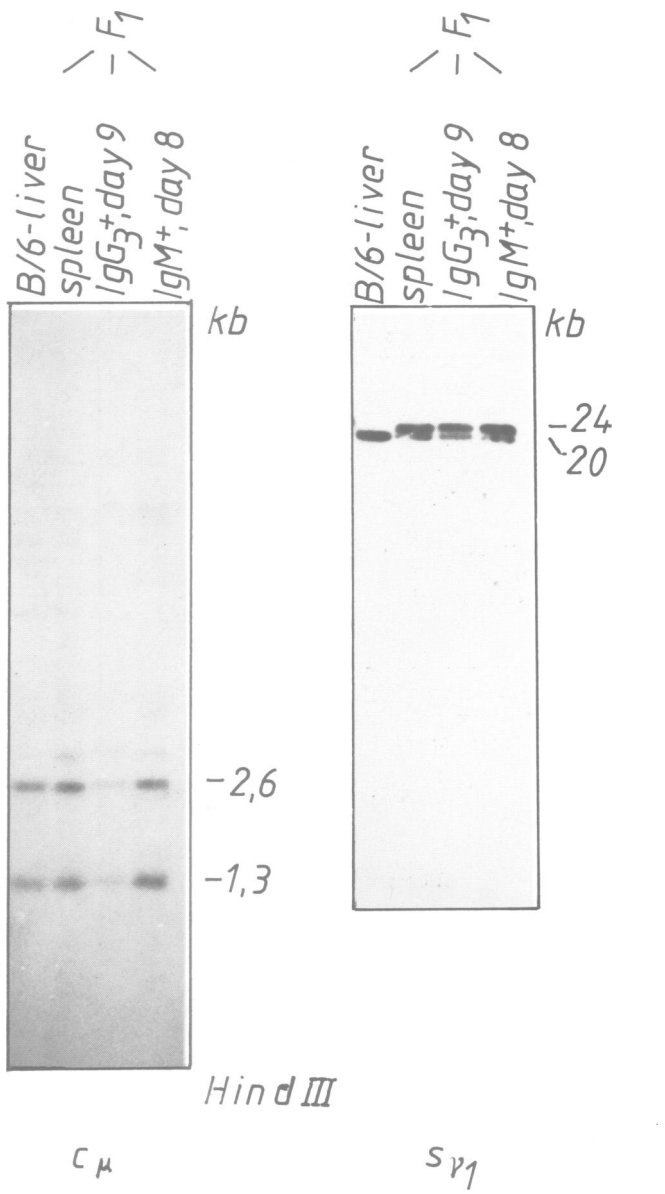

Fig. 5. DNA of surface IgM^+ 8-day-old F_1 LPS blasts, 9-day-old surface $IgM^-/IgG3^+$ F_1 blasts and C57BL/6 liver cells was cut with *Hind*III and analysed as described in Figure 4.

a probabilistic model of class switching. According to this model the Cμ gene would be deleted in most of the LPS blasts from the active or inactive chromosome without preference and eventually from both chromosomes. The blast cell continues to express IgM if Cμ is deleted from the inactive chromosome. If Cμ is deleted from the active chromosome, the cell switches to expression of another Ig class. Such cells would still contain one Cμ gene. $sIgG3^+$ cells from 6-day-old cultures could be a subpopulation of these cells. Later in the course of LPS stimulation Cμ is then sometimes deleted from the previously affected chromosome in cells that have already deleted it from one chromosome. Regardless of whether the first deletion happened on the inactive or active chromosome, i.e., the cell expressing Cμ or another C_H gene, it will now have no Cμ gene left and so will express another Ig class. An example of such a population would be $sIgG3^+$ blasts from 9-day-old cultures. Most of the 8 day $sIgM^+$ blasts would have deleted Cμ from the inactive chromosome.

At present, we cannot decide between the two models described since we cannot discriminate the alleles of IgM and IgG3 on a serological basis and thus are not able to select appropriate cells to distinguish between deletions on active and inactive chromosomes. The second model, however, appears to us to be more attractive because of its simplicity and because it is analogous to the probabilistic model for V/D/J joining as proposed by Coleclough *et al.* (1981).

Materials and methods

Animals

Mice were obtained from our own breeding colony.

Cell culture and panning

Spleen cells of 5–10 (C57BL/6 x BALB/c)F1 mice were pooled and treated with tris-ammonium chloride (Boyle, 1968). Dead cells were removed by filtration through cotton wool columns. Cells were seeded in 500 ml Falcon culture flasks at $0.5-2 \times 10^6$/ml in 50–100 ml RPMI 1640, 10% fetal calf serum (FCS), 2×10^{-5} M 2-mercaptoethanol, penicillin (100 U/ml), streptomycin (100 μg/ml), gentamicin (50 μg/ml) and 40 μg/ml LPS (*Escherichia coli* O55:B5 or *E. coli* O111:B4, Sigma, Munich, FRG). On days 2 or 3 and 4, 5 or 6 the cultures were fed by addition of 50–100 ml fresh

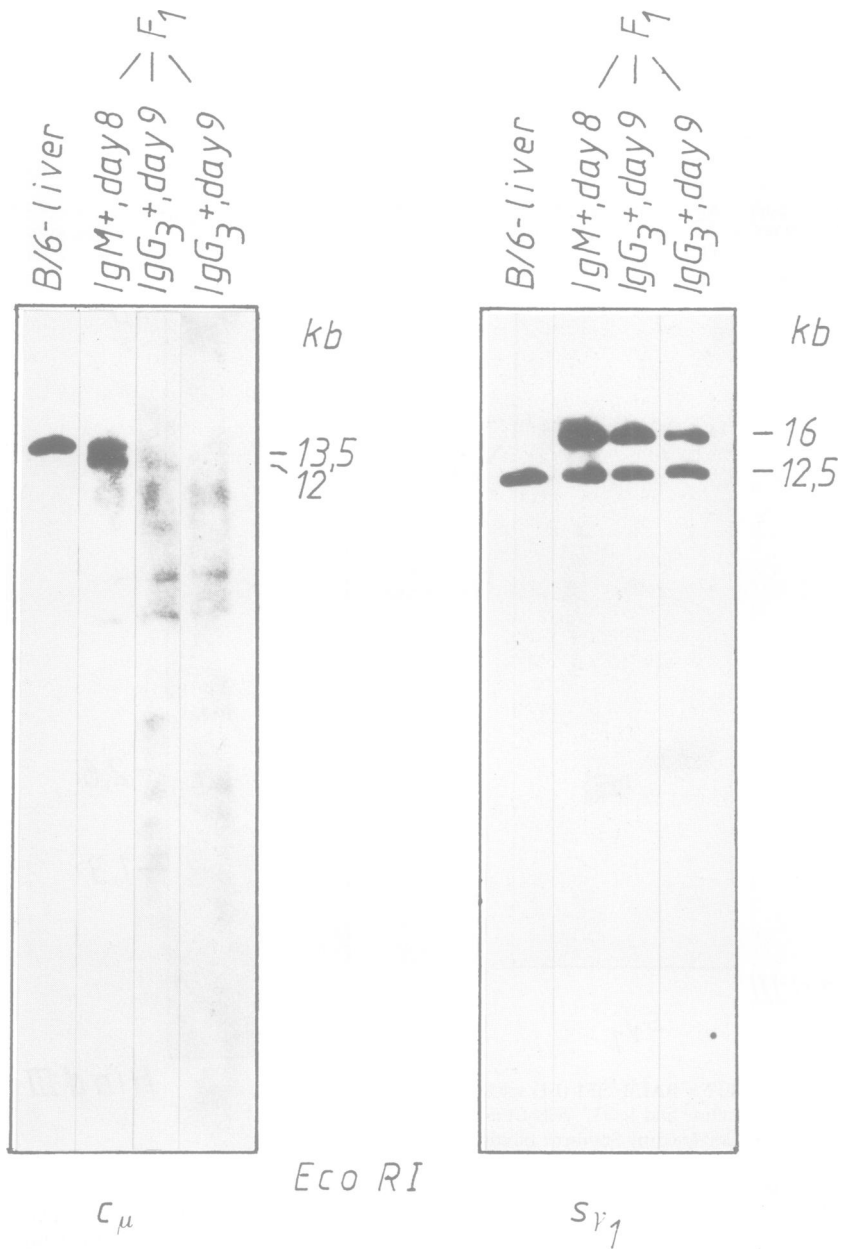

Fig. 6. DNA of C57BL/6 liver cells, F_1 sIgM$^+$ day 8 LPS blasts and day 9 sIgG3$^+$ F_1 LPS blasts was cut with *Eco*RI, hybridised first to the Cμ-specific probe and then to the sγ1 probe. Both, the Cμ and sγ1 germline *Eco*RI fragments are of different sizes for the a and b haplotypes of the IgH locus. In order to demonstrate the faint Cμ band in **lane 3**, **lanes 3** and **4** of the Cμ hybridisations are overexposed compared with **lanes 1** and **2**.

medium with LPS. On days 5 or 8 the cells were harvested and live cells purified on Ficoll-isopaque (Mohr and Krawinkel, 1976) by centrifugation at 1000 *g* for 10 min at room temperature. For panning, 18 cm diameter Petri dishes (Greiner, Nürtingen, FRG) were coated with 100–200 μg/ml affinity-purified goat anti-mouse IgM, saturated with Dulbecco's phosphate buffered saline (DPBS) with 5% FCS and the cells applied at 5 x 10^6/ml and 10 ml/dish in DPBS, 5% FCS and 0.03% azide twice for 20 min, gently resuspending unbound cells after the first 20 min. Unbound cells were gently removed and transferred to medium with LPS. Bound cells were removed with a rubber policeman and used for preparation of DNA.

Immunofluorescence and cell sorting

For fluorescence microscopy the cells were double stained for surface Ig with fluorochrome-conjugated goat anti-isotype antibodies at 4°C for >30 min in the presence of 0.03% azide, washed, spun on slides in a cytocentrifuge (Shandon, UK) covered with Elvanol or dried, fixed in 95% ethanol/5% acetic acid and stained for cytoplasmic Ig (Kearney and Lawton, 1975; Kearney *et al.*, 1976).

For cell sorting, live cells that had been re-stimulated for 1 day after panning were purified over Ficoll-isopaque if necessary and stained for surface Ig with affinity-purified goat anti-IgM and anti-IgG3 antibodies conjugated wih Texas red and fluorescein, respectively, as previously described (Kearney *et al.*, 1976; Titus *et al.*, 1982). For sorting, the cells were washed, diluted to 0.5–2 x 10^7/ml with 10 μg/ml propidium iodide added (Sigma, Munich, FRG) and filtered through a nylon mesh with 30 μm mesh width. Cell sorting was done in a modified FACS I (Becton-Dickinson, Sun Valley, CA, B. Liesegang *et al.*, in preparation) with a 4 W argon laser and a rhodamine 6G dye laser. Deflected cells were collected in Eppendorf tubes and counted. An aliquot was used for preparation of a control slide and the rest spun down and used for preparation of high mol. wt. DNA.

Restriction endonuclease analysis

The DNA of the various cell populations was analysed by the Southern hybridisation technique (Southern, 1975; Wahl *et al.*, 1980) using the enzymes and probes described in Figure 3. The Cμ-specific probe was kindly provided by A. Bothwell and the sγ1 probe by T. Honjo. The sγ1 hybridisation was more effective than the Cμ hybridisation. This, the decay of the label of the Cμ probe and the dissociation of the Cμ probe from the filter during hybridisation with sγ1 explains, why Cμ bands are hardly ever visible on sγ1 autoradiographs.

The relative amount of Cμ in the various LPS blast populations was estimated by densitometric scanning of autoradiographs of the Southern blots with monochromatic green light in a Quick Scan Jr. slit scanner (Helena Lab., DESAGA, Heidelberg, FRG). The linear dose-response range of the film (Kodak X-ray) was determined by exposing a linear stepwise grey scale to the film and comparing the exposure with the scale itself and the autoradiograph. For comparison of band intensities we used only autoradiographs where the peaks were in the linear part of the dose-response curve of the film as determined with the grey scale. Within this range the peak-height was directly proportional to the amount of radioactivity on the filter because, on multiple films derived from the same filter, the peak height increased linearly with the time of exposure (data not shown). Background subtraction was performed only for background of the film, not for background of individual lanes. We could not detect patchy background hybridisation on the filters analysed except for the filter shown in Figure 4B. As can be seen in that case, the patches did not extend over the area of the peaks. The quantitative analysis was not considerably influenced by the shape of the peaks. Comparision of peak areas and peak heights gave similar results. We preferred the peak heights as a measure because parts of the peak profiles were not in the linear dose-response range of the film. The heights of the sγ1 peaks were taken as a measure for the relative DNA content per lane. The ratio of sγ1 and Cμ peak heights thus gives an estimate of the fraction of Cμ genes deleted or rearanged per cell population.

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