The EMBO Journal vol.8 no.2 pp.483 - 488, 1989

Rapid induction of transcription of unrearranged $s_\gamma 1$ switch regions in activated murine B cells by interleukin 4

Charlotte Esser and Andreas Radbruch

Institut für Genetik der Universität zu Köln, Weyertal 121, D-5000 Köln 41, FRG

Communicated by K.Rajewsky

The distribution of immunoglobulin isotypes in activated B lymphocytes can be modulated by interleukin 4 (IL4), which enhances IgG1 and suppresses IgG3. We show here that IL4 induces transcription of the region 5' adjacent to the $s_\gamma 1$ switch region within hours after onset of activation of B cells by bacterial lipopolysaccharide (LPS). Transcripts of 1.7 and 3.2 kb size containing sequences of the region 5' of $s_\gamma 1$ are detected. This transcription precedes class switch recombination between s_μ and $s_\gamma 1$ and reflects the rapid opening of the $s_\gamma 1$ region as induced by IL4. This suggests that IL4 directs class switching to IgG1 by opening the $s_\gamma 1$ switch region, thus making it accessible for switch recombination.

Key words: accessibility model/immunoglobulin class switch/interleukin 4/transcription

Introduction

The distribution of immunoglobulin (Ig) isotypes (classes) in an immune response may vary depending on the nature of the antigen, its presentation and the kind of T cell help involved (Rosenberg, 1982). Polyclonal activators such as bacterial lipopolysaccharide (LPS) mimic antigenic stimulation in that they induce proliferation, secretion of Ig and immunoglobulin class switching at high frequency. Murine splenic B cells stimulated with LPS preferentially switch from IgM to IgG3 (Coutinho and Forni, 1982; Kearney and Lawton, 1975). In the presence of the T cell lymphokine interleukin 4 (IL4), however, the cells switch predominantly to IgG1 instead of IgG3 (Layton *et al.*, 1984). Cells switching to IgG1 are committed to do so, as can be deduced from the fact that the majority of them have performed switch recombination between s_μ and $s_\gamma 1$ switch regions on both the active and the inactive IgH locus (Radbruch *et al.*, 1986; Winter *et al.*, 1987).

It is not clear when (before or after activation) and how a B cell becomes committed to switch to a particular class. Switch recombination could be directed to distinct switch regions by isotype-specific recombinases (Davis *et al.*, 1980), or the 'accessibility' of immunoglobulin switch regions for recombination could be under specific control (Stavnezer-Nordgren and Sirlin, 1986; Alt *et al.*, 1986). In support of the latter model, in some B cell lines specific transcription of unrearranged switch regions of certain classes accompanies switch recombination directed to those Ig classes (Stavnezer-Nordgren and Sirlin, 1986; Alt *et al.*, 1986; Lutzker and Alt, 1988). Here we analyse whether transcription of unrearranged $s_\gamma 3$- and $s_\gamma 1$-switch regions is induced by LPS or LPS plus IL4 in murine splenic B cells and whether this transcription is correlated to and occurs before class switching to IgG3 and IgG1, i.e. whether IL4 controls class switching by regulating the 'accessibility' of switch regions for recombination.

Results

Experimental strategy

RNA was prepared and analysed from murine spleen cells activated with LPS alone or with LPS and IL4 at various time points after onset of stimulation. Total cellular RNA was used to include in the analysis transcripts that do not leave the nucleus. The stimulations were controlled for frequency of switched plasmablasts at days 5 or 6. Transcription of unrearranged switch regions was assayed by quantitating transcripts containing sequences 5' of the $s_\gamma 3$ or $s_\gamma 1$ switch regions. Such transcripts cannot be generated from IgH loci after class switch recombination between s_μ and $s_\gamma 3$ or $s_\gamma 1$ because the 5' sequences are deleted by the recombination (Winter *et al.*, 1987). We first determined the relative abundance of transcripts of various immunoglobulin heavy chain (C_H) genes in normal spleen cells and then analysed the increase of transcripts during LPS and LPS plus IL4 induction. Northern blots were done to analyse the sizes and ratios of transcripts.

Specificity of probes and hybridization assays

The probes used in this study were controlled for cross-reactivity on Southern blots of restriction enzyme digested DNA of various murine IgH haplotypes (not shown). $5's_\gamma 1$ and $5's_\gamma 3$, the two most critical probes, do not cross-react with each other. They hybridize strongly to the expected germline fragments. $5's_\gamma 3$ detects very weakly an unknown sequence that does not have the fragment size that would be expected from any of the other probes used here. In addition, all probes were hybridized to slot blots of total RNA from several myeloma and hybridoma lines (Table I). The RNA of many cell lines contains transcripts of various parts of their Ig-loci. We do not want to discuss here the possible significance of this finding but have used it to test for nonspecific hybridization in slot blots. Equal amounts of poly(A)$^+$ RNA were applied to the slot blots, hybridized to the various probes and the quantitated hybridization signals evaluated. In none of the cell lines $5's_\gamma 1$ could transcripts be detected. The $5's_\gamma 3$ and $5's_\gamma 1$ probes do not detect 'productive' $_\gamma 3$ or $_\gamma 1$ transcripts from active IgH loci with $s_\mu/s_\gamma 3$ and $s_\mu/s_\gamma 1$ switch recombinations because $5's_\gamma 3$ does not react with RNA from the IgG3 Producer 64.22 ($s_\mu/s_\gamma 3$ on both IgH loci) and $5's_\gamma 1$ does not detect $_\gamma 1$ transcripts of the IgG1 expressing hybridoma G8H4 ($s_\mu/s_\gamma 1$ on both IgH loci). Thus, both probes do not react with $C_\gamma 1$ sequences, as also shown in Figure 4. Table I shows in

Table I. Relative RNA-slot blot signals

Cell line	Presence/absence of DNA-regions 1	2	3	4	C_μ (1)	$5's_\gamma3$ (2)	$5's_\gamma1$ (3)	$C_\gamma1$ (4)
PC140[a] (IgM)	■	■	■	■	++++	−/+	−	−
64.22 (IgG3)	□	□	■	■	−	−	−	−
G8H4 (IgG1)	□	□	□	■	−	−	−	++++
LMTK⁻ fibroblast	■	■	■	■	−	−	−	−
Spleen cells					+/−	+	−/+	−/+
LPS blasts	38 h				+++	++	−/+	+
LPS + IL4 blasts	38 h				+	++++	++++	+

[a]Total RNA.

Test for cross-reactivity of the probes used in this study. Equal amounts of poly(A^+) RNA of several cell lines were hybridized to the indicated probes. Autoradiographs of slot blots that had been exposed for the same lengths of time were compared and the strengths of the hybridization signal evaluated from '++++' (strong) to '−' (no signal) in 20% intervals, relative to the strongest signal obtained. Beside each cell line the presence (black box) or absence (empty box) of C_μ (1), $5's_\gamma3$ (2), $5's_\gamma1$ (3) and $C_\gamma1$ (4) sequences in the genome of these lines is noted.

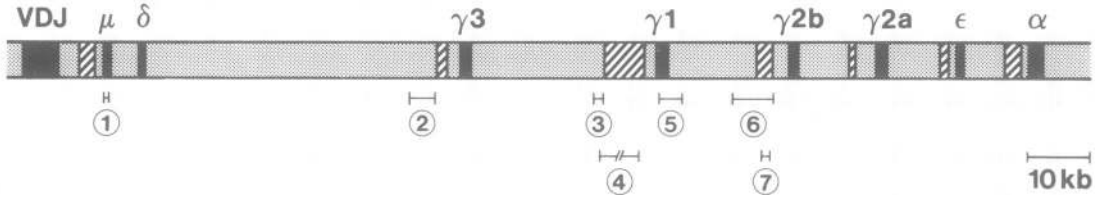

Fig. 1. Map of the immunoglobulin heavy chain locus of an IgM B cell drawn according to Shimizu *et al.* (1982). Black boxes indicate the C_H-genes; switch regions are hatched. Probes are drawn as bars. (1) C_μ; (2) $5's_\gamma3$; (3) $p5's_\gamma1$; (4) $ps_\gamma1$; (5) $C_\gamma1$; (6) $'s_\gamma2b$; (7) $s_\gamma2b$ probe.

addition that there is no unspecific hybridization due to high concentration of RNA on the nitrocellulose.

Overall increase of RNA production

We find that the overall RNA content of spleen cells activated by LPS increases about 10-fold from about 10 $\mu g/10^7$ cells at the onset of stimulation to about 20 $\mu g/10^7$ cells at day 1 and further up to 100 $\mu g/10^7$ cells at day 5 of LPS stimulation. The percentage of poly(A^+) RNA remains constant at 1−2%.

Abundance of $5's_H$ and C_H transcripts in the spleen

The relative abundance in the spleen of transcripts of C_H and 5′ switch regions is approximated in slot blots using the probes listed in Figure 1. Serial dilutions were prepared from the DNA probes, applied to nitrocellulose and hybridized to the same probes. The hybridization signals derived from these titrations were plotted as shown in Figure 2 as DNA concentration versus signal intensity. The values obtained with RNA of spleen or hybridoma cells on the same blot were plotted on the best fit straight lines of the DNA titrations to show the relative abundance of $5's_\gamma3$, $5's_\gamma1$, $C_\gamma1$ and C_μ transcripts in unactivated spleen cells. C_μ transcripts are the most abundant ones. Compared to IgM secreting hybridoma cells, however, spleen cells contain an average of only about 1% of C_μ transcripts per cell, and 0.25% of $C_\gamma1$ transcripts compared to IgG1 secreting hybridoma cells, originating presumably from the few IgG1 plasmablasts in spleen. Transcripts including sequences 5′ of switch regions or of switch regions themselves are ~10- ($5's_\gamma3$) to 100-fold ($5's_\gamma1$) less frequent in splenic B cells than C_μ transcripts. Whereas $5's_\gamma3$ transcripts are detectable in slot blots, $5's_\gamma1$ transcripts remain barely detectable even after long exposure times. $5's_\gamma3$ transcripts are more than one order of magnitude more frequent than $5's_\gamma1$ transcripts (Figure 2).

Induction of transcription of the S_H and C_H regions after activation with LPS and IL4

The induction of transcription along the Ig-C_H locus is shown in Figure 3. Equal amounts of RNA (5 μg) isolated from variable numbers of cells were applied to each slot. RNA hybridizing to the various probes is quantitated relative to the signal of RNA from unstimulated spleen cells with that probe, which is set to 1.0. Thus the specific rather than the overall increase of transcription is plotted. Compared to unstimulated cells, cells stimulated with LPS and IL4 contain more transcripts of C_μ and $C_\gamma1$. The increase is moderate (C_μ) or not detectable ($C_\gamma1$) within the first 38 h, but drastic for C_μ and $C_\gamma1$ after day 3, when IgM and IgG1 secreting cells appear (Jones *et al.*, 1985). C_μ transcripts are more prominent in LPS stimulated cells, in agreement with previously published work (Jones *et al.*, 1985; Snapper and Paul, 1987), while $C_\gamma1$ transcripts are much more abundant in LPS plus IL4 blasts than in LPS blasts, corresponding to the different frequency of IgG1 expressing cells in these cultures. A rapid increase in number of transcripts is observed for the $5's_\gamma1$ and $5's_\gamma3$ probes (Figure 3a). In LPS plus IL4 blasts at 9 h after onset of stimulation there are 3-fold and at 38 h 20-fold more $5's_\gamma1$ transcripts than in unstimulated spleen cells. In LPS blasts the frequencies of these transcripts increase only moderately. Thus, transcription of the $5's_\gamma1$ region starts before the first cell divisions. Previous work from other authors shows that the transition from the G_0 to the G_1 phase, as judged by DNA

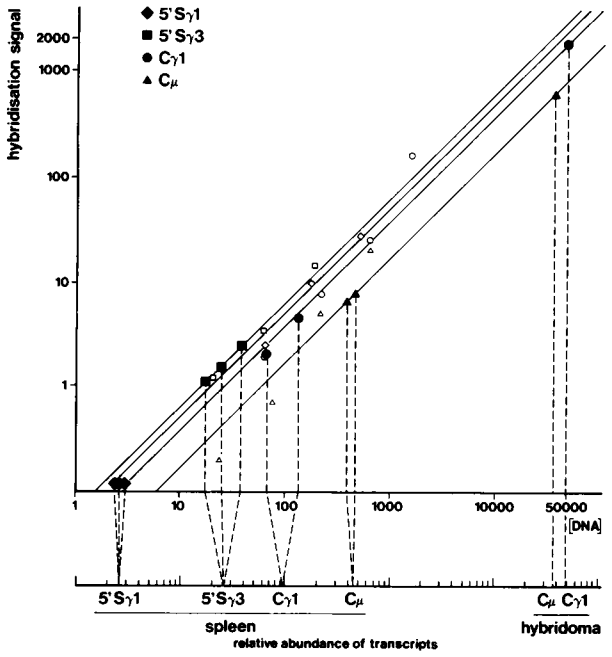

Fig. 2. Relative abundance of 5's_γ1, 5's_γ3 and C_γ1, C_μ-transcripts in splenic B cells. The intensity of the hybridization signal obtained with poly(A^+) RNA from 2×10^8 spleen cells (large closed symbols) was plotted relative to the best fit line in correlation with the signals given by the cold probe itself (small open symbols), titrated on the same slot blot. The signal obtained from the total RNA of hybridoma lines 267.7μ and G8H4 was extrapolated. RNA and DNA were treated identically as described in Materials and methods.

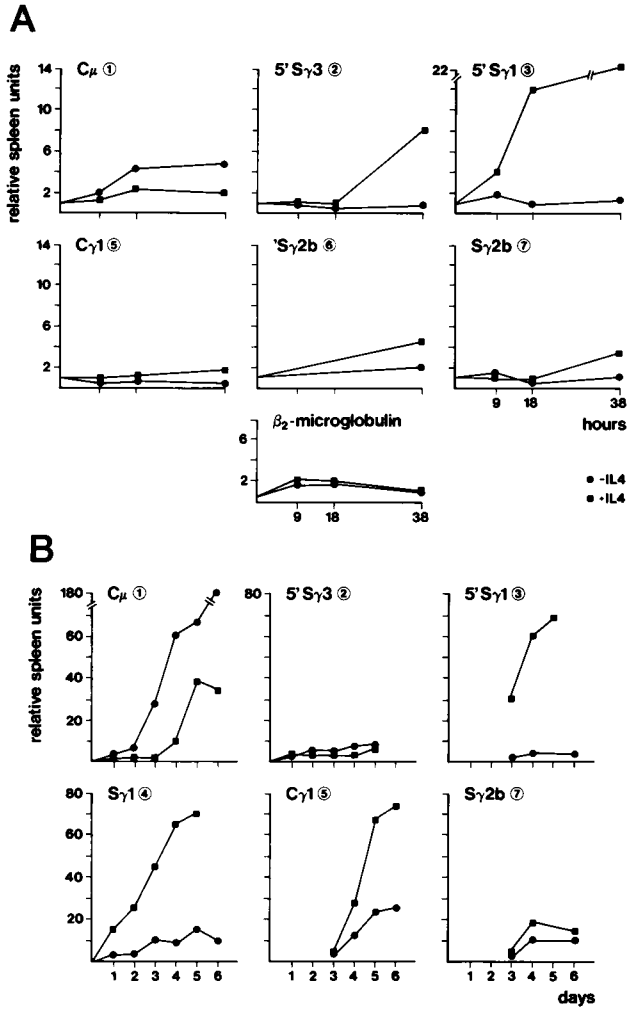

Fig. 3. Slot blot analysis of the specific increase of the transcription of IgH locus sequences. The amount of transcripts detected with a particular probe is plotted relative to the abundance of these transcripts in unstimulated spleen cell RNA which is set to 1.0. For abundance of 5's_γ1, 5's_γ3, C_γ1 and C_μ transcripts in spleen cells see Figure 2. (a) 0, 9, 18 and 38 h, (b) 1–6 days stimulated with LPS (●) or LPS plus IL4 (■). 5 μg of poly(A^+) RNA (a) or total RNA (b) were blotted per slot and the filter hybridized successively to the different probes. Sequence of probes: 5's_γ1, C_γ1, s_γ2b, 5's_γ3, C_μ and β_2-microglobulin. Each probe was controlled on the same filter with RNA from cell lines. The data of 's_γ2b are derived from a different slot blot.

and RNA synthesis and cell diameter, begins at ~10 h after exposure to LPS (Pike *et al.*, 1987; Leanderson *et al.*, 1987). Also 5's_γ3 transcripts are more frequent in LPS plus IL4 blasts than in LPS blasts and unstimulated spleen cells, although the difference is not drastic. 5's_γ3 transcripts increase ~8-fold within the first 38 h of LPS plus IL4 stimulation. For 5's_γ3 in LPS stimulations a similar increase is sometimes observed, at most 5-fold within 48 h (Figure 3b). 's_γ2b/s_γ2b transcripts increase slightly in LPS plus IL4 cultures whereas no increase is detected with LPS only. The increase of s_γ transcription is due to B cells because the same increase is observed in cultures derived from B cells purified by panning on anti-IgM (data not shown).

Size of 5's_γ transcripts

The 5's_γ1 and 5's_γ3 transcripts of activated B cells are characterized according to size by Northern blotting as shown in Figure 4. Transcripts hybridizing to the 5's_γ1 probe are 1.7 and 3.2 kb in size. In accordance with the slot blot analysis they occur in increasing amounts over the time of LPS plus IL4 stimulation, first detectable after 24 h. The Northern blotting technique used here is less sensitive than the slot blot technique, thus the initial increase in the slot blot analysis is below the level of detection. The C_γ1 probe detects equal amounts of the 1.7 (secretory) and 3.2 kb (membrane) C_γ1 transcripts in spleen cells until day 2 of stimulation with LPS or LPS plus IL4. After that the 1.7 kb transcript becomes dominant; at day 5 at least 40-fold more 1.7 than 3.2 kb transcripts are found, in parallel with the increase in IgG1 secreting plasmablasts. In contrast, the 5's_γ1 transcripts of 1.7 and 3.2 kb size are present in equal amounts for the 5 days of stimulation. They do not follow the differential regulation of secretory and membrane forms of $_\gamma$1 chains in IgG1 secreting cells. The 5's_γ1 probe does not detect the functional $_\gamma$1 mRNA because it does not hybridize to RNA from G8H4, an IgG1 secreting hybridoma that has deleted all DNA upstream of the s_γ1 region. The smaller C_γ1-transcripts seen with X63Ag8.653 are derived from the remaining non-productive C_γ1 gene in this immunoglobulin non-producer cell line. The 5's_γ3 probe, like the 5's_γ1 probe, detects transcripts of 1.7 and 3.2 kb size in roughly equal amounts in cells stimulated with LPS or LPS plus IL4 (Figure 4). The 5's_γ3 probe does not hybridize to the 5's_γ1 probe (Figure 4, left lane; see also above).

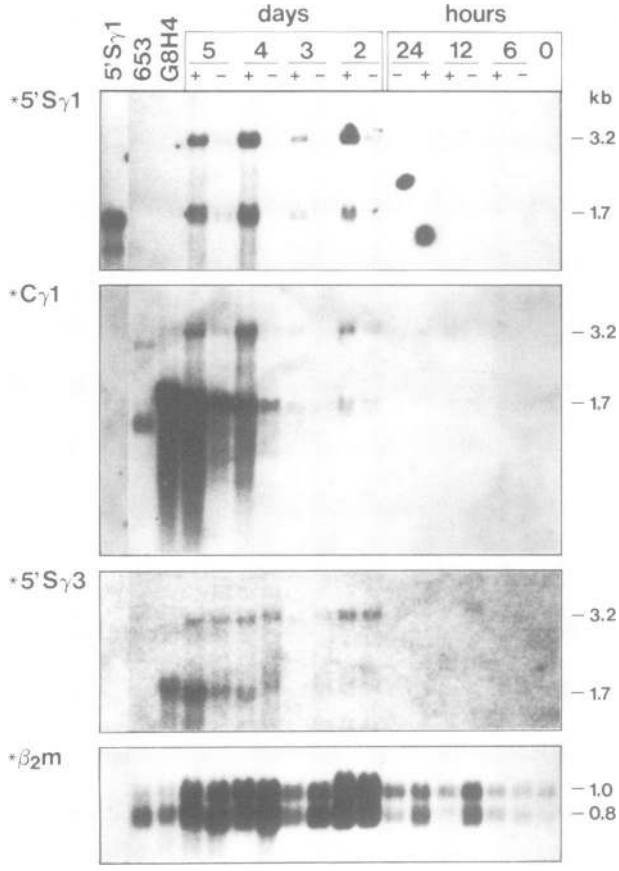

Fig. 4. Northern blot of RNA from naive and LPS and LPS plus IL4 stimulated splenic B cells, the cell lines X63Ag8.653 and G8H4 and DNA of the $5's_\gamma1$ probe, hybridized sequentially to the (**a**) $5's_\gamma1$, (**b**) $C_\gamma1$, (**c**) $5's_\gamma3$ and (**d**) β_2-microglobulin probe (to show the amount of RNA loaded). Autoradiographic exposure times differ for each probe. $5's_\gamma1$ and $C_\gamma1$ transcripts both increase with time, albeit from a different baseline (see Figure 2). $5's_\gamma1$ transcripts are detectable first on day 2. $C_\gamma1$ transcripts do not increase between the early hours and day 3, LPS plus IL4 (equal β_2-microglobulin signal); the signal is probably mostly derived from VDJ-$C_\gamma1$ transcripts of IgG^+ cells. Comparing day 2 to days 4 and 5 (equal β_2-microglobulin signal), an increase of $C_\gamma1$ transcripts is obvious (see Figure 3), mostly of the secretory form (1.7 kb). The 1.7 kb signal apparently obtained with the $5's_\gamma3$ probe on G8H4, and LPS plus IL4, day 5, is a relict of the $C_\gamma1$ hybridization (only this signal) as was determined by autoradiography.

Discussion

One of the biologically important features of immunoglobulin class switching is the regulated distribution of isotypes in an immune response. Only few data are available on induction of class switching and molecular events involved in committing a cell to switch to a particular class. Here we study the induction of class switching in normal B-lymphocytes activated with LPS. In the presence of IL4 class switch recombination is directed to $s_\gamma1$ rather than to $s_\gamma3$, which is the predominant switch in cells activated with LPS alone. How can recombination be targeted to distinct switch regions? It has been postulated that the accessibility of a switch region for a common switch recombinase is regulated in a specific way (Alt *et al.*, 1986). 'Accessibility' could be considered as 'open chromatin', measurable in terms of DNase I hypersensitivity, demethylation or transcription. Transcripts of parts of the IgH locus have been found in transformed B cells which showed recombination involving those parts of the IgH locus (Blackwell *et al.*, 1986; Stavnezer-Nordgren and Sirlin, 1986). In order to test whether the T cell derived lymphokine IL4 can induce transcripts of the $s_\gamma1$ switch region in activated B cells, we have quantitated transcripts derived from various switch regions prior to switch recombination in the presence and absence of IL4. Indeed, upon activation of B cells with LPS and IL4, but not with LPS alone, transcripts of the $s_\gamma1$ switch region sequence are found in increasing amounts. This process is quite rapid: in the presence of IL4 the amount of transcripts from unrearranged $s_\gamma1$ switch regions triplicates within hours. In addition to the 20-fold increase in the amount of $s_\gamma1$ transcripts, an 8- and 4-fold increase of transcripts containing $s_\gamma3$ and $s_\gamma2b$ sequences respectively is induced by IL4 within 38 h. At first glance this seems to contradict the accessibility model since switch recombination to $s_\gamma3$ and $s_\gamma2b$ is less frequent in LPS plus IL4 blasts than in LPS blasts although $5's_\gamma3$ transcripts are not more abundant in the latter. This apparent contradiction may be resolved by the observation that even before the onset of B cell activation about 10 times more $5's_\gamma3$ than $5's_\gamma1$ transcripts are found in spleen cells. Thus, considering the frequency of switch region transcripts and measure for 'openness' of the regions in naive LPS activated B cells, $s_\gamma3$ is open but $s_\gamma1$ closed in these cells. In B cells activated with LPS and IL4 the $s_\gamma1$ switch region is opened as well. In this case $s_\gamma1$ (located 3' to $s_\gamma3$), is apparently the preferred target of switch recombination.

The present data clearly show that induction of $s_\gamma1$ transcripts by IL4 precedes switch recombination directed to $s_\gamma1$ in these cultures. It remains to be shown whether transcription or transcripts play a role in the process of switch recombination. The process of transcription opens the chromatin, therefore the induction of transcription could make it accessible for switch recombination. The transcripts could be mere by-products or play a direct role. Since RNA as part of catalytic ribonucleoproteins has been described in other systems, e.g. the telomerase found in *Tetrahymena* (Dreyfuss *et al.*, 1988; Greider and Blackburn, 1987).

The homogeneous size of the germline s_γ transcripts as determined by Northern blots suggests that RNA synthesis may start from a distinct promoter upstream of the $s_\gamma1$ or $s_\gamma3$ switch region and that the 3' parts of the transcripts are likely to be the C_γ gene in both its membrane and secreted form. This would resemble the situation described by Lutzker and Alt (1988). They found a pseudo-exon upstream of $s_\gamma2b$ spliced onto the $C_\gamma2b$ gene in AMuLV transformed pre-B cells.

It is still an open question where $5's_\gamma$ transcripts start. One might speculate that the effect of IL4 is simply to prolong transcription of the $5's_\gamma3$ region downstream to $s_\gamma1$. The observed transcripts would then be distinct splice products of a common precursor. It has been postulated that before class switch recombination B lymphocytes can express two isotypes simultaneously via splice products of a primary transcript spanning large parts of the IgH locus (Perlmutter and Gilbert, 1984; Chen *et al.*, 1986). Such a primary transcript could be the precursor of $5's_\gamma$ transcripts.

It will be interesting to analyse how IL4 induces B-cells to $s_\gamma1$ transcription. This activation may exemplify how

joining and class switch recombination in the course of lymphocyte differentiation are regulated by external (or internal) signals.

Materials and methods

Mice

Female (BALB/c × C57BL/6)F_1 mice were obtained from our own breeding colony.

LPS cultures

Spleen cells were cultured in RPMI 1640 supplemented with 10% FCS and antibiotics in the presence of LPS (40 μg/ml, Sigma, St Louis, MO). Supernatant of CHO cells containing recombinant IL4 (Müller, 1987) was added at a concentration of 10%. The cells were fed and diluted every 2 or 3 days. Aliquots of all cultures were maintained at least until day 6 and the quality of the stimulations was controlled by cytoplasmic staining of the cells from cultures at day 6 with isotype-specific fluorescent antibodies and determination of the frequencies of switched plasmablasts (Radbruch, 1986). Only LPS cultures with 5–10% IgG3 and <1% IgG1 cells and LPS plus IL4 cultures with 15–30% IgG1 and <1% IgG3 cells were used.

Preparation of RNA

Total RNA was prepared according to the guanidinium isothiocyanate/cesium choride method of Chirgwin *et al.* (1979). As a modification of their protocol, the cell lysate was passed through an 18 gauge syringe to shear chromosomal DNA. Concentration of the RNA was determined by measuring at OD_{260}. To get rid of possible contamination with DNA, the RNA was digested with RNase-free DNase (Boehringer, Mannheim, FRG). Poly(A^+) RNA was purified over oligo(dT) columns according to standard procedures (Maniatis *et al.*, 1982).

Slot blotting and Northern blotting

RNA was denatured in 12% formaldehyde/20 × SSC (3 M NaCl, 0.3 M sodium citrate) and blotted onto BA-45 nitrocellulose membranes with a suction apparatus (Minifold II, Schleicher and Schüll, FRG). The membranes were air dried, baked for 2 h at 80°C and hybridized to ^{32}P-labeled probes in 5 × SSC, 0.4% bovine serum albumin, 0.4% Ficoll 400, 0.4% polyvinylpyrrolidone, 500 μg/ml denatured herring sperm DNA and 10% dextran sulfate at 65°C. Washes were performed in 3 × and 2 × SSC/1% SDS for 30 min. The filters were rehybridized after removing the probe by washing the filter in H_2O/1% SDS at 65°C and confirming the removal by autoradiography. For Northern blotting the RNA was electrophoretically fractionated in 1% agarose gels containing 6% formaldehyde in 20 mM MOPS, 5 mM sodium acetate and 1 mM EDTA. The RNA was blotted directly onto nylon membranes (Genescreen$^+$, NEN research products, Boston, USA) in 20 × SSC. As a size marker *Hin*dIII restricted λ phage DNA was used. The filters were hybridized overnight in 1 M NaCl, 50 mM Tris (pH 7.0), 10% dextran sulfate and 500 μg/ml denatured herring sperm DNA. Filters were washed with 3 × and 2 × SSC/1% SDS for 30 min. Hybridized slot and Northern blots were autoradiographed at −70°C to pre-flashed Kodak X-AR5 films using intensifier screens.

Densitometry

The extent of RNA induction was determined by scanning the autoradiographs with a densitometer (Desaga Quickscan, Heidelberg,FRG) at 525 nm. Quantitations are derived from autoradiographs within the linear range of radioactivity to photosensitivity. The resulting values are depicted as multiples of the signal found for unstimulated spleen cells with that probe on that filter.

Probes

All probes were used as purified inserts except the 's_γ2b probe which was cloned into pUC9. Probes were labelled to average sp. act. of $5 \times 10^8 - 1 \times 10^9$ c.p.m./μg DNA either by nick translation (Rigby *et al.*, 1977) or by random primer labeling (Feinberg and Vogelstein, 1983). We used a panel of probes that cover relevant parts of the immunoglobulin locus (Figure 1).

(i) C_μ(pC$_\mu$TS1). 1 kb *Aval*/*Pst*I cDNA fragment from pABμ11 (gift of A.Bothwell, Bothwell *et al.*, 1981), spanning C_{H1}–C_{H3} and subcloned into pUC8.

(ii) *5's_γ3*. 4.2 kb *Eco*RI/*Bgl*II fragment cut out from pJW6 (Wels *et al.*, 1984). The distance between the 3' end of this fragment and the start of the s_γ3 49-mer consensus sequence (Stanton and Marcu, 1982) is ^{400} bp.

(iii) *5's_γ1 (p5's_γ1BglII)*. 1.6 kb *Bgl*II fragment starting directly upstream of the s_γ1 region. The fragment was subcloned from p$_\gamma$1E/H10.0 (Mowatt and Dunnick, 1986) into the *Bam*HI site of pTZ19R.

(iv) *s_γ1:(ps$_\gamma$1)*. 5 kb *Eco*RI fragment of the γ1 switch region in pUC9 (Shimizu *et al.*, 1982).

(v) *C$_\gamma$1:(pG1A)*. 3.5 kb genomic *Eco*RI/*Bam*HI segment containing half of the C_H1 domain of C_γ1 and extending to 3'UT (Shimizu *et al.*, 1982).

(vi) *'s_γ2b*. A 6.6 kb germline *Eco*RI fragment, containing the s_γ2b switch region and sequences 5' thereof (Hummel *et al.*, 1987).

(vii) *s_γ2b:(ps$_\gamma$2b)*. 1.4 kb *Eco*RI fragment in pBR322, constructed by K.Marcu (unpublished).

(viii) *β_2-Microglobulin*. 0.7 kb cDNA clone of β_2-microglobulin (Morello *et al.*, 1982).

Acknowledgements

We thank K.Rajewsky, I.Haas, U.Krawinkel and the Cologne class switch group for stimulating discussions and critical reading of the manuscript. We are grateful to W.Müller for preparation of recombinant IL4. This work was supported by a grant from the Bundesministerium für Forschung und Technologie (Genzentrum Köln, group 2.07).

References

Alt,F.W., Blackwell,T.K., DePinho,R.A., Reth,M.G. and Yancopoulos,G.D. (1986) *Immunol. Rev.*, **89**, 5–30.
Andersson,J., Coutinho,A. and Melchers,F. (1977) *J. Exp. Med.*, **145**, 1511–1519.
Blackwell,T.K., Moore,M.W, Yancopoulos,G.D., Suh,H., Lutzker,S., Selsing,E. and Alt,F.W. (1986) *Nature*, **324**, 585–589.
Bothwell,A.L.M., Paskind,M., Reth,M., Imanishi-Kari,T., Rajewsky,K. and Baltimore,D. (1981) *Cell*, **24**, 625–637.
Chen,Y.-W., Word,C., Jones,S., Uhr,J.W., Tucker,P.W. and Vitetta,E.S. (1986) *J. Exp. Med.*, **164**, 548–561.
Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry*, **18**, 5294–5299.
Coutinho,A. and Forni,L. (1982) *EMBO J.*, **1**, 1251–1257.
Davis,M.M., Kim,S.K. and Hood,L. (1980) *Science*, **289**, 1360–1365.
Dreyfuss,G., Philipson,L. and Mattaj,I.W. (1988) *J. Cell. Biol.*, **106**, 1419–1425.
Feinberg,A.P. and Vogelstein,B. (1983) *Anal. Biochem.*, **132**, 6–13.
Greider,C.W. and Blackburn,E.H. (1987) *Cell*, **51**, 887–898.
Hummel,M., Berry,J.K. and Dunnick,W. (1987) *J. Immunol.*, **138**, 3539–3548.
Jones,S., Layton,J., Krammer,P.H., Vitetta,E.S. and Tucker,P.W. (1985) *J. Mol. Cell. Immunol.*, **2**, 143–153.
Kearney,J.F. and Lawton,A. (1975) *J. Immunol.*, **115**, 671–676.
Lang,R.B., Stanton,L.W. and Marcu,K.B. (1982) *Nucleic Acids Res.*, **10**, 611–630.
Layton,J.E., Vitetta,E.S., Uhr,J.W. and Krammer,P.H. (1984) *J. Exp. Med.*, **168**, 1850–1863.
Leanderson,T., Andersson,J. and Rajasekar,R. (1987) *Immunol. Rev.*, **99**, 53–69.
Lutzker,S. and Alt,F.W. (1988) *Mol. Cell. Biol.*, **8**, 1849–1852.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York.
Morello,D., Daniel,F., Baldacci,P., Cayre,Y., Gachelin,G. and Kourilsky,P. (1982) *Nature*, **296**, 260–262.
Mowatt,M.R. and Dunnick,W.A. (1986) *J. Immunol.*, **136**, 2674–2683.
Müller,W. (1987) Ph.D. Thesis, Cologne, FRG.
Perlmutter,A.P. and Gilbert,W. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 7189–7194.
Pike,B.L., Alderson,M.R. and Nossal,G.J.V. (1987) *Immunol.Rev.*, **99**, 19–52.
Radbruch,A., Müller,W. and Rajewsky,K. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 3954–3957.
Radbruch,A. (1986) In Weir,D.M. (ed.), *Handbook of Experimental Immunology*. Blackwell Scientific Publications, Oxford, Vol. 4.

Rigby,P.W., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237–251.
Rosenberg,Y.J. (1982) *Immunol. Rev.*, **67**, 33–58.
Shimizu,A., Takahashi,N., Yaoita,Y. and Honjo,T. (1982) *Cell*, **28**, 499–506.
Snapper,C.M. and Paul,W.E. (1987) *J. Immunol.*, **139**, 10–17.
Stanton,L.W. and Marcu,K.B. (1982) *Nucleic Acids Res.*, **19**, 5993–6006.
Stavnezer-Nordgren,J. and Sirlin,S. (1986) *EMBO J.*, **5**, 95–102.
Wels,J.A., Word,C.J., Rimm,D., Der-Balan,G.P., Martinez,H.M., Tucker,P.W. and Blattner,F.R. (1984) *EMBO J.*, **3**, 2041–2046.
Winter,E., Krawinkel,U. and Radbruch,A. (1987) *EMBO J.*, **6**, 1663–1671.

Received on September 19, 1988