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Regulation of *Ig* Gene Expression in Murine B-Lymphocytes *

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A. Introduction

B cells at different stages of maturation exhibit distinct patterns of *Ig* gene transcription. Most of the information we have about those patterns is derived from tumour systems. Using B myelomas as models for pre-B cells, B lymphomas for early B cells and plasmacytomes as analogues of fully differentiated plasma cells, several groups have reported that the rate of transcription across the heavy-chain locus differs only slightly in these various lines and that the steady-state levels of heavy-chain mRNA are predominantly regulated by post-transcriptional events [7, 8]. In contrast, Yuan and Tucker (1984) [14], who investigated the heavy-chain transcription in resting normal B cells and in B cells stimulated with LPS for 4 days, described an eight- to tenfold increase in the rate of transcription. This increase is smaller than that observed in the amount of steady-state μ-specific mRNA upon LPS stimulation.

B. Stimulation of μ and κ Transcription After LPS Stimulation

To evaluate the relative contributions of transcriptional and post-transcriptional regulation of both H and L chains at various times in normal B-cell development, we studied B cells activated either by LPS alone or by LPS together with anti-*Ig* antibodies. The latter model system was chosen in order to gain some understanding of the events which might take place in situations in which the Ag receptor is occupied by the relevant antigen. Heavy- and light-chain transcription was studied by nuclear run on assays, and the transcription rates were related to the amounts of steady-state mRNA for the κ chain and for the membrane and secreted forms of the μ chain.

Our data showed that after LPS stimulation of normal B cells, the amounts of both μ and κ are regulated at the level of transcription [4]. Transcriptional activation is accompanied by μ_m–μ_s transition. The increase in the transcription rates (30- to 60-fold) quite faithfully reflects the increase in steady-state μ and κ mRNA (30- to 100-fold); delta expression, on the other hand, seems to be negatively regulated at the post-transcriptional level. This is inferred from the observation that although transcription across the delta locus did not terminate after stimulation, no mature mRNA was detectable.

C. Post-transcriptional Regulation of μ and κ Expression in Cells Co-stimulated with LPS and anti-*Ig* Antibodies

While LPS stimulation of B cells thus clearly leads to high levels of *Ig*-relevant mRNA, stimulation with anti-μ or anti-κ alone, which induces proliferation, has no such ef-

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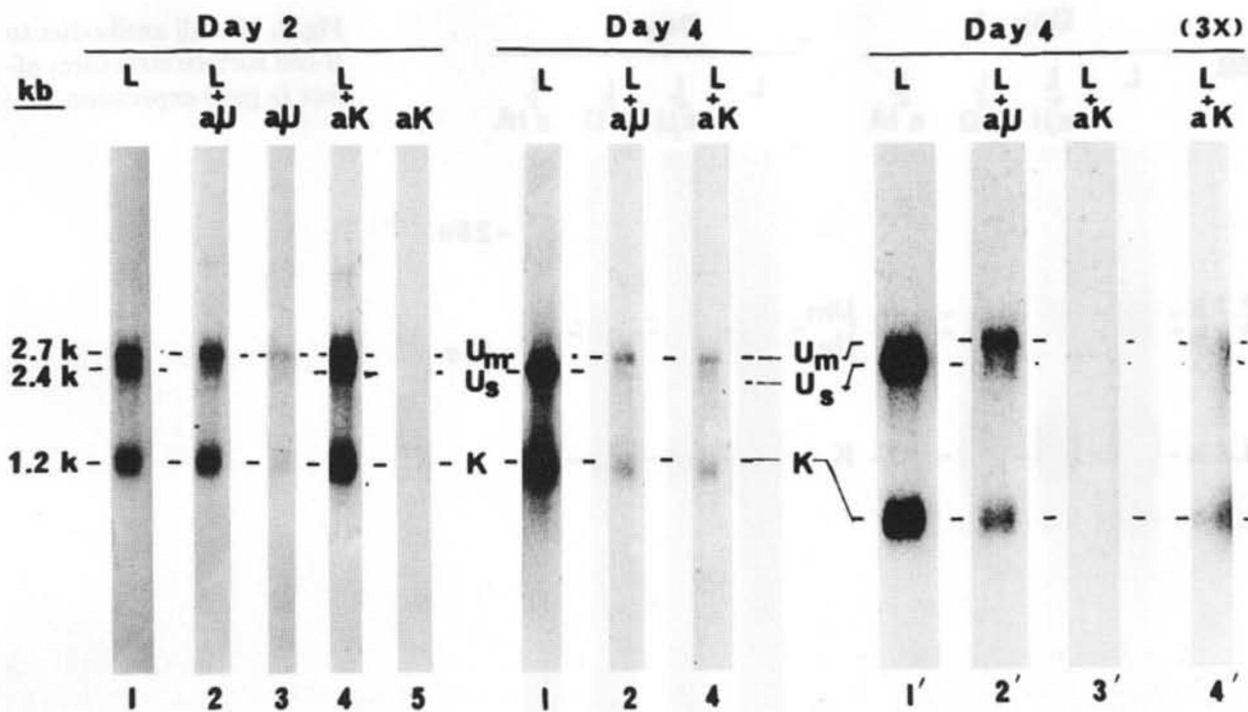

Fig. 1. Co-stimulation of B cells with anti-μ/anti-κ and LPS leads to profound effects on H- and L-chain mRNAs. For cell preparations and RNA analyses see [3]

fect. Indeed, it has been found that co-stimulation of normal B cells with LPS and $F(ab')_2$ fragments of antibodies to μ and κ decreases LPS-induced *Ig* secretion while high levels of proliferation are maintained [1, 3].

We studied the effect of co-stimulation of LPS and anti-receptor antibodies on the steady-state mRNA levels for μ and κ (Fig. 1). It was possible to draw the following conclusions: (a) anti-μ or anti-κ treatment by itself does not lead to mRNA levels higher than those observed in resting cells (resting cells not shown) even though, under the conditions used in the experiment, the cells incorporate thymidine and have been shown to undergo at least one cell cycle. (b) LPS plus anti-μ treatment leads to increased μ_m and κ mRNA levels on day 2, comparable to those observed with LPS alone, but to no μ_s mRNA, which makes up approximately 50% of the total μ mRNA detected in LPS-stimulated cells at that time. LPS plus anti-κ-treated cells on day 2 show high levels of both μ_m/μ_s and κ mRNAs. (c) In either case, on day 4, μ_s, μ_m and κ mRNA levels are very low in all doubly treated cells, while B cells stimulated with LPS alone show the μ and κ levels characteristic of that state of B-cell development [10, 3]. Treatment with anti-μ or anti-κ $F(ab')_2$ fragments affects the mRNA levels of both chains, i.e. even those not directly recognized by the antibody. Non-*Ig*-related gene expression such as *H-2* is not affected (data in [3]).

Using nuclear run-on assays, we were able to show that the loss of μ and κ mRNAs is due not to cessation of transcription but mostly to post-transcriptional events which affect stability and/or processing of the H- and L-chain RNA [3].

Not all antibodies to B-cell surface structures affect *Ig* gene expression equally. Figure 2 shows that antibodies to the delta chain of the *Ig* receptor and to *I-A* do not affect LPS-induced mRNA levels to the same extent as anti-μ or anti-κ. When RNA was analysed on day 2, LPS plus anti-I-A-stimulated cells exhibited an mRNA pattern identical to that observed with LPS alone. Analysis on day 4 again showed co-stimulation with anti-I-A to have little or no effect. Co-stimulation with anti-delta does lead to a certain reduction in μ-specific mRNA. Transition to μ_s is somewhat delayed (see day 4 as compared to LPS controls), but not prevented. Since these experiments were performed with total resting splenic B cells, the weaker suppression by anti-delta might be due to the fact that only 30%–50% of these

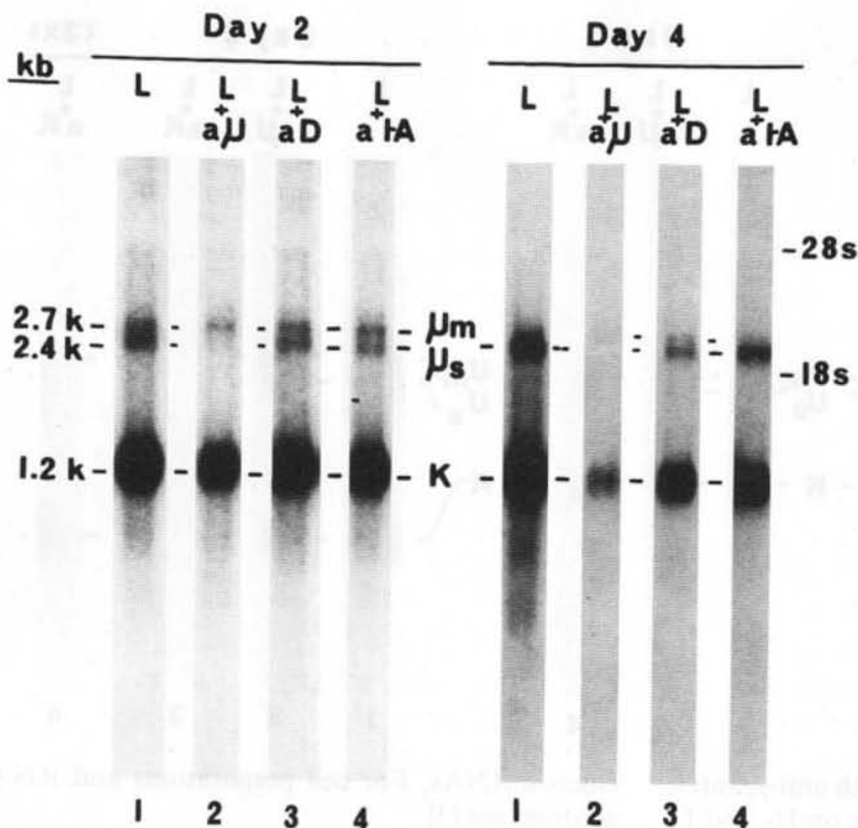

Fig. 2. Not all antibodies to B-cell surface structures affect *Ig* gene expression

cells carry delta on the surface and that the delta-negative cells can be stimulated by LPS. If this is so, the data also imply that only cells directly interacting with anti-*Ig* antibodies exhibit downregulation of *Ig* expression; this argues against an indirect effect mediated by some unknown suppressor mechanism.

D. Post-transcriptional Downregulation Induced by anti-μ Antibodies Cannot be Reversed by Cycloheximide

Recently, several systems have been described in which mRNA levels are post-transcriptionally regulated. In several of these systems, e.g. *c-myc* [5], *c-fos* [11] and *Il-2* [6], cycloheximid can stabilize mRNA levels without affecting transcription [6]. In the pre-B-like 70Z cells, cycloheximide treatment has in addition been shown to increase κ gene transcription [13]. We therefore attempted to influence or stabilize μ-mRNA levels in LPS plus anti-μ co-stimulated cells. Figure 3 shows that contrary to the systems described above, cycloheximide has very little, if any, effect. This would suggest that the mechanism of post-transcriptional con-

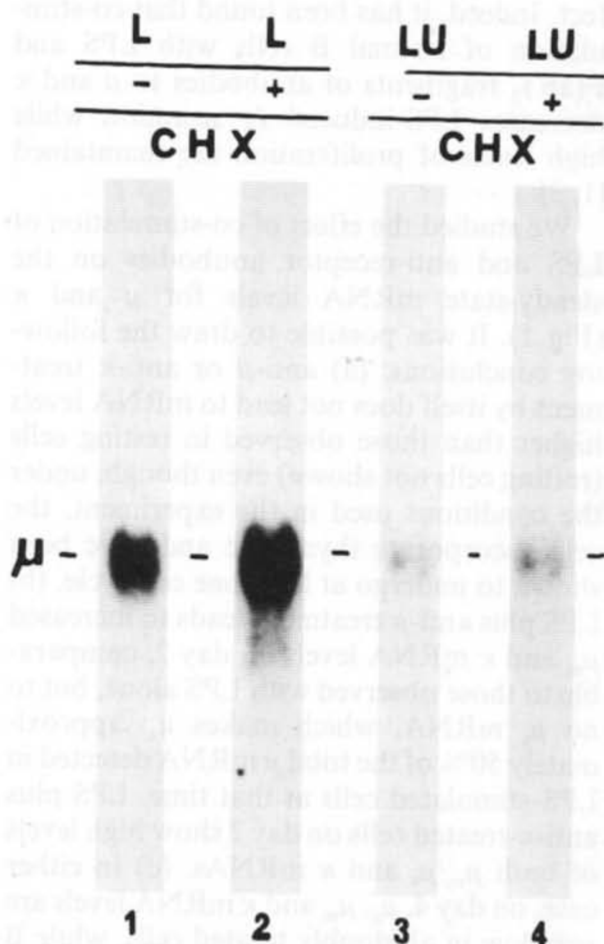

Fig. 3. Lack of mRNA stabilization after treatment of single or doubly stimulated cells with cycloheximide. *L*, LPS; *LU*, LPS + anti-μ

trol operative in the LPS plus anti-μ system is different from that observed with c-*myc*, c-*fos* and *Il-2*.

E. Signals Which May Mediate Anti-*Ig*-Induced Downregulation

Binding and cross-linking of anti-μ and anti-κ to the Ag receptor leads to receptor shedding and endocytosis. It also leads to activation of the phosphatidyl-inositol pathway, resulting in inositol triphosphate and diacylglycerol formation, and thus in the mobilization of intracellular Ca^{2+} and the activation of protein kinase C [2]. To investigate whether the endocytosed antibodies or the signal induced by them are responsible for *Ig*-mRNA downregulation, we replaced the receptor-specific antibodies with phorbol esters and the Ca-ionophore ionomycin in the co-stimulation with LPS. Table 1 shows that this treatment closely mimics that of antibody treatment with respect to both sustained proliferation and inhibition of *Ig* secretion, at least up to day 3–4 of culture. If these observations can be substantiated by molecular analysis of gene transcription and mRNA accumulation, they would suggest that endocytosis of the antibodies to the receptors is not obligatory. However, these experiments do not rule out the possibility that the endocytosed receptor itself might mediate the negative effect, since treatment of B cells with ionomycin and phorbol esters leads to rapid disappearance of IgM from the surface (data not shown).

Table 1. Inhibition of *Ig* secretion in B cells stimulated with LPS and phorbolmyristate acetate plus ionomycin

Treatment of cells	cpm/5×10^4	PFC/5×10^4
Med	794	18
LPS	15041	2000
LPS+PMA +ionomycin	19330	36
PMA+ionomycin	11774	25

Resting splenic B cells were isolated and stimulated with either LPS (10 µg) or phorbolmyristate acetate (5 ng) plus ionomycin (0.5 µM), or with a combination of both reagents. Cultures were pulsed with 0.25 µCi of ^{3}H-thymidine for 16 h on day 3 or assayed for polyclonal Ig secretion on day 4, using a modified reverse plaque assay [12]. PFC, plaque-forming cells.

F. Possible Relevance of *Ig*-RNA Downregulation for B-Cell Physiology

Continued proliferation of B cells with concomitant downregulation of *Ig* gene expression might be valuable for the generation of memory cells. Downregulation of *Ig* secretion prevents the cells from reaching the end-stage of plasma cells, while continued proliferation might allow for the events leading to *Ig* class switch to take place. To investigate this possibility we established hybridomas from B cells either stimulated with LPS alone or with LPS plus anti-μ $F(ab')_2$. The two types of cells were fused on days 4 and 5 after stimulation; hybridomas were established and analysed for the Ig class formed. Table 2 shows the results. They indicate that both LPS and LPS plus anti-μ stimulated cells give rise to *Ig*-secreting hybridomas, i.e. anti-μ-induced inhibition of *Ig* secretion is reversible. The data also show that fusion on day 5 leads to hybridomas producing *Igs* other than IgM and that these occur more frequently in the doubly stimulated cells than in those stimulated by LPS alone. The hybridomas obtained after LPS plus anti-Ig stimulation might lead to a valuable insight into the mechanism of the class switch which in normal B cells, in addition to other signals, may be favoured by a transient post-transcriptional downregulation of *Ig* gene expression such as has been found in the model system described here.

Table 2. IgG-producing hybridomas from LPS or LPS+$\mu F(ab')_2$-stimulated B cells

Day of fusion	LPS	LPS+$\mu F(ab')_2$
4	0/24	0/12
5	4/70	12/60

Resting B cells were isolated and activated as described in [3]. On the days indicated, hybridomas were established [9].

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