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Cytokine-induced differentiation of IgA B cells: studies using an IgA expressing B-cell lymphoma

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SUMMARY

Cytokines such as interleukin-5 (IL-5) and transforming growth factor β1 (TGFβ1) increase IgA production by heterogeneous populations of lipopolysaccharide (LPS)-activated murine B cells. We have used IgA expressing murine B-lymphoma cells CH12.LX.C4.4F10 (4F10) to define the activity of these and other cytokines on IgA secretion at the single-cell level, membrane IgA expression, IgA polymerization and cell growth. IL-5 as well as LPS significantly increases IgA secretion of 4F10 cells, whereas TGFβ1, a cytokine known to stimulate isotype switching to IgA among surface IgM-bearing B cells, inhibits IgA secretion. When tested alone, IL-1β, IL-2, IL-4, IL-6 and interferon-gamma (IFN-γ) do not significantly alter IgA secretion. However, there is a synergistic increase in IgA secretion when 4F10 cells are co-stimulated with IL-5 and IL-4, while IFN-γ inhibits IL-5-stimulated up-regulation of IgA secretion. In parallel with increased IgA secretion after cytokine stimulation, 4F10 cells display less membrane IgA. Increased J-chain steady-state mRNA levels after IL-5 or LPS stimulation are paralleled by increased mRNA levels for secreted IgA, but are not accompanied by alterations in the ratio of monomeric to polymeric IgA. IL-5 and LPS initially stimulated but later inhibited 4F10 cell proliferation suggesting an inverse relationship between proliferation and differentiation in this cell line. 4F10 cells are a useful model for the characterization of discrete aspects of IgA B-cell differentiation, since the secretory and membrane Ig and proliferative responses of this IgA B-cell line to cytokines and LPS appear to parallel those of freshly isolated murine B cells.

INTRODUCTION

IgA is the predominant immunoglobulin in mucosal secretions. High-level IgA secretion is a function of mucosal IgA plasma cells which develop from membrane IgM-expressing B cells. *In vitro* studies with lipopolysaccharide (LPS)-activated, freshly isolated murine B cells have identified several cytokines that affect one or several steps in the development of IgA B cells.[1–13] However, studies of IgA B-cell growth and differentiation using freshly isolated murine B cells have several limitations. Thus, freshly isolated B cells are heterogeneous with respect to their state of activation and differentiation. This has made it difficult to define the exact IgA B-cell functions that are influenced by specific cytokines. Moreover, freshly isolated B cells require activation for survival in culture and the activators added to culture can, themselves, alter B-cell growth, differentiation and response to cytokines. Finally, it is difficult to obtain sufficient numbers of homogeneous populations of freshly isolated IgA-bearing B cells to characterize the molecular mechanisms that underlie specific aspects of IgA B-cell differentiation. To circumvent these problems we have used a homogeneous population of IgA-expressing murine B lymphoma cells CH12.LX.C4.4F10 (4F10)[14,15] to study the effects of cytokines and LPS on distinct aspects of IgA B-cell growth and differentiation. We show here that 4F10 cells respond to physiological agonists in several aspects (IgA secretion, membrane IgA expression and growth characteristics) in a manner similar to that observed with freshly isolated spleen B cells, making this cell line a useful model for defining molecular events that underlie discrete steps in IgA B-cell differentiation.

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MFI, median fluorescence intensity; mIg, membrane immunoglobulin.

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MATERIALS AND METHODS

Cytokines and other reagents

The following cytokines, known to be active on murine B cells, were used in these studies: recombinant human (rh) interleukin-1β (IL-1β) (Genzyme Corporation, Boston, MA), rhIL-2 (Cellular Products Inc., Buffalo, NY), recombinant murine (rm) IL-4 (Genzyme), rmIL-5 (a gift from Dr K. Takatsu, Kunamoto University, Japan), rmIL-6 (Genzyme), rm interferon-gamma (IFN-γ) (Genentech, South San Francisco, CA) and porcine

transforming growth factor β1 (TGFβ1) (R&D Systems, Minneapolis, MN). Rabbit anti-TGFβ1 was obtained from R&D Systems. TRFK-5, a rat monoclonal antibody to murine IL-5[16] was a gift from Dr R. L. Coffman (DNAX, Palo Alto, CA). Bacterial LPS (*Escherichia coli*, 0111:B4) was obtained from Sigma Chemical Co. (St Louis, MO).

Cell line and cell culture

The CH12.LX.C4.4F10 cell line is a membrane IgA$^+$ spontaneous clonal switch variant of the *in vitro* adapted parental line CH12.LX[15] (kindly provided by Dr G. Haughton, University of North Carolina, NC). Studies in our laboratory have shown that, like the parental cell line CH12,[17] 4F10 cells express κ light chains (data not shown), and Southern blot analysis of 4F10 cells has indicated that both immunoglobulin heavy chain loci have undergone isotype switch recombination to C_α (data not shown). Cells were kept in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS), 5 mM HEPES, 2 mM glutamine, 40 μM 2-mercaptoethanol and antibiotics. Cell densities were maintained between 10^4/ml and 10^6/ml, since rapid cell death occurred above a cell density of $1{\cdot}5 \times 10^6$/ml. Cell viabilities, as assessed by trypan blue dye exclusion, were greater than 90% in all experiments.

ELISA and ELISPOT assays

ELISA and enzyme-linked immunospot (ELISPOT) assays were performed as described previously.[5,8] IgA secretion is presented as IgA concentration in the culture supernatants divided by cell density, and is expressed as nanograms of IgA secreted per 10^6 cells.

Flow cytometric analysis

Cells were stained with 2 μg/ml fluorescein isothiocyanate (FITC)-labelled goat anti-mouse IgA antibody (Southern Biotechnology, Birmingham, AL) for 30 min at 4°, fixed in 1% formalin in phosphate-buffered saline (PBS) and analysed on a flow cytometer (FACScan; Becton Dickinson, Mountain View, CA). Gating for viable cells was performed using forward and perpendicular light scatter signals.

Analysis of IgA by ultracentrifugation

Two hundred microlitres of test samples was applied on top of linear gradients (4·8 ml) of 5–20% sucrose in PBS pH 7·3 in 5 ml polyallomer tubes and centrifuged at 200,000 ***g*** for 7 hr at 20°. Fractions of 200 μl were collected and assayed for α heavy chains by ELISA.

Northern blot analysis

RNA was extracted using the guanidine isothiocyanate/cesium chloride method, size-fractionated on an agarose/formaldehyde gel and transferred to nitrocellulose.[18] The following probes, labelled with ^{32}P, were used for hybridization: a 660 base pair (bp) *Pvu*II fragment of pα(J558), a murine cDNA clone carrying sequences present in both the secretory and membrane α heavy chain constant region[19] (a gift of Dr P. Tucker, University of Texas, Dallas, TX); a 2·8 kb *Hin*dIII/*Bam*HI fragment of pHBC$_\kappa$, a plasmid containing murine immunoglobulin κ light chain constant region[20] (a gift from Dr. S. Desiderio, John Hopkins University, Baltimore, MD); a 1·24 *Xba*I fragment of pJcX containing a murine J chain cDNA (provided by Dr M. E. Koshland, University of California, Berkeley, CA); a 1·2 kb *Pvu*II fragment of pRβA-1, a rat β-actin cDNA clone (a gift from Dr L. Kedes, University of Southern California, Los Angeles, CA); a 780 bp *Pst*I/*Xba*I fragment of pHcGAP, a human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA clone (ATCC, Rockville, MD).[21] After hybridization, blots were washed under stringent conditions (0·1 × SSC, 0·1% SDS, 60°, 2 × 20 min) and exposed to X-ray film (Kodak, X-omat AR). Bound radioactivity was quantitated using a radioanalytic imaging system (Ambis Systems, San Diego, CA).

Data analysis

Data analysis used a modified version of Student's *t*-test which does not assume equal variances of the two test groups.[22] Calculations were performed with the statistical software package SYSTAT (Systat Inc., Evanston, IL).

RESULTS

IgA secretion by 4F10 cells is increased by IL-5 or LPS, and decreased by TGFβ1

To test the ability of several cytokines implicated in the regulation of the IgA response, and of LPS, to alter IgA secretion by 4F10, cells were incubated with the cytokines or with LPS for 3 days, after which the amount of IgA secreted into the culture supernatants was determined by ELISA. To separate cytokine effects on IgA secretion from those on cell growth, IgA concentration in the culture supernatants was divided, for each sample, by the final cell density in the cultures.

As shown in Table 1, LPS stimulation of 4F10 cells increased IgA secretion by approximately 20-fold, while cell growth was only slightly, but significantly, stimulated during the 3-day culture period. Stimulation of 4F10 cells with IL-5 increased IgA secretion approximately four-fold and cell proliferation slightly. Specificity of the IL-5 activity was confirmed by the addition of an anti-IL-5 monoclonal antibody (TRFK-5) to the cultures, which completely blocked the IL-5-stimulated increase in IgA secretion. Addition of IL-1, IL-2, IL-4, IL-6 or IFN-γ had no significant effect on IgA secretion by 4F10 cells.

In contrast to LPS or IL-5, TGFβ1 (0·1 ng/ml) decreased the secretion of IgA by 4F10 cells approximately 40% and also inhibited cell proliferation. Higher concentrations of TGFβ1 (up to 1·6 ng/ml) did not decrease IgA secretion further, although they further inhibited 4F10 cell proliferation. An anti-TGFβ antibody completely blocked the effects of TGFβ1 on both the secretion and proliferation of 4F10 cells. IFN-γ, which also inhibited 4F10 cell proliferation, did not inhibit IgA secretion.

The effects of various combinations of cytokines and LPS on IgA secretion and cell proliferation of 4F10 cells were also tested (Table 1). The combination of IL-4 and IL-5 resulted in a synergistic increase in IgA secretion compared to cells stimulated with IL-5 or IL-4 alone. In contrast, addition of IFN-γ to IL-5-stimulated cultures inhibited the increase in IgA secretion induced by IL-5 to less than 50% of that seen in cultures stimulated with IL-5 alone. Combinations of IL-2, IL-4, IL-6 and IFN-γ had no significant effect on IgA secretion by 4F10 cells (data not shown).

IgA secretion, as reported above, reflects the accumulation of IgA in the cultures over a 3-day period, but only indirectly reflects the actual secretory state of 4F10 cells at the end of the

Table 1. Effect of various cytokines and LPS on IgA secretion by 4F10 cells*

Additions to culture†	Cell density cells/ml ($\times 10^3$)	IgA secretion ng/10^6 cells	IgA secretion Fold increase over unstimulated controls
None	900±599	1332±88	1·00±0·07
LPS	1199±58‡	27384±2019‡	20·56±1·52‡
IL-1β	1063±298	1566±311	1·18±0·23
IL-2	1038±88	1442±127	1·08±0·10
IL-4	944±97	1732±371	1·30±0·28
IL-5	1197±78‡	5828±574‡	4·38±0·43‡
IL-6	973±275	1636±285	1·23±0·21
IFN-γ	476±102‡	1885±632	1·42±0·47
TGFβ1	575±85‡	754±70‡	0·57±0·05‡
LPS+IL-2	1210±160	29319±6032	22·01±4·53
LPS+IL-4	1203±256	23199±5156	17·42±3·87
LPS+IL-5	1297±167	32627±5942	24·49±4·46
LPS+IL-6	1218±58	22903±4303	17·19±3·23
LPS+IFN-γ	829±174	28792±4829	21·62±3·63
LPS+TGFβ1	1336±150	23751±2190	17·83±1·64
IL-5+IL-2	1279±222	8283±1359	6·22±1·02
IL-5+IL-4	992±174	14382±939§	10·80±0·70§
IL-5+IL-6	944±188	3326±706	2·50±0·53
IL-5+IFN-γ	535±69§	2494±342§	1·87±0·26§
IL-5+TGFβ1	753±279	4087±1294	3·07±0·97
TGFβ1+IL-2	735±150	1405±583	1·05±0·44
TGFβ1+IL-4	724±138	623±122	0·47±0·09
TGFβ1+IL-6	532±160	684±111	0·51±0·08
TGFβ1+IFN-γ	142±24¶	685±106	0·51±0·08

* 4F10 cells (2×10^4/ml) were cultured for 3 days with LPS and/or various cytokines as indicated. Cell densities and IgA concentrations in culture supernatants were determined at the end of the culture period. IgA secretion is given as IgA concentration in the culture supernatants divided by cell density. Results are means±SEM of three or more experiments.

† Cytokines and LPS were tested over a range of concentrations (given below in brackets) and were used at the following optimal concentrations: LPS, 10 μg/ml (0·04–50 μg/ml); IL-1β, 200 pg/ml (4–200 pg/ml); IL-2, 25 IU/ml (1–500 IU/ml); IL-4, 5 ng/ml (0·75–7·5 ng/ml); IL-5, 100 U/ml (1·6–400 U/ml); IL-6, 10 ng/ml (1·3–160 ng/ml); IFN-γ, 4 ng/ml (0·04–1024 ng/ml); TGFβ1, 0·1 ng/ml (0·008–1·6 ng/ml).

‡ Values significantly different from medium control, $P<0·01$.

§ Values significantly different from IL-5 alone, $P<0·01$.

¶ Values significantly different from TGFβ1 alone, $P<0·01$.

stimulation period. To characterize the secretory state of 4F10 cells directly, cells were cultured for 3 days with LPS, IL-5 or TGFβ1, washed and replated at varying cell densities for 2 hr, after which the amount of IgA secreted into the culture supernatant was assayed (Table 2). Cells that were stimulated with LPS or IL-5 for the previous 3 days, secreted approximately 10- or fourfold more IgA, respectively, during the 2-hr culture period than control cells. In contrast, cells preincubated with TGFβ1 for 3 days secreted 40% less IgA during the 2-hr culture period. Moreover, the amount of IgA secreted over the 2-hr culture period was proportional to the number of plated cells (data not shown). Taken together, these results indicate that the final IgA concentration divided by the final cell density, after 3 days in culture, provides a reliable estimate of the secretory state of the 4F10 cell line.

Increased IgA secretion by 4F10 cells stimulated with LPS or IL-5 could reflect the recruitment of non-secreting 4F10 cells to IgA secretion or an increase in IgA secretion per cell. To distinguish between these possibilities, the proportion of IgA-secreting cells in unstimulated or stimulated 4F10 cultures was determined by ELISPOT assay. All 4F10 cells were shown to secrete IgA before and after stimulation with LPS or IL-5. However, LPS- or IL-5-stimulated cells formed spots of increased size in the ELISPOT assay (Fig. 1), indicating that 4F10 cells secreted an increased quantity of IgA per cell. TGFβ1 inhibited IgA secretion by 4F10 cells. However, here also, ELISPOT analysis revealed that each cell secreted IgA.

Increased IgA secretion by 4F10 cells is accompanied by decreased expression of membrane IgA

To examine the effect of cytokines and LPS on the expression of membrane IgA (mIgA), 4F10 cells were cultured with the different cytokines or LPS for 3 days and analysed for mIgA expression by flow cytometry. Differences in mIgA expression among unstimulated and stimulated cell populations were compared by dividing the median fluorescence intensity (MFI) of stimulted cells and the MFI of unstimulated cells (Table 3). Stimulation with LPS and IL-5 decreased mIgA expression per cell by approximately 55 and 30%, respectively. Other cytokines (IL-1, IL-2, IL-4, IL-6, IFN-γ or TGFβ1) did not significantly alter mIgA expression of 4F10 cells. Thus, following either cytokine or LPS stimulation, mIgA expression is inversely correlated with IgA secretion. Moreover, the decrease in total mIgA expression per cell after stimulation, as measured by MFI, reflects a decreased cell surface density of mIgA, since the cell size of unstimulated and stimulated 4F10 cells was identical, as assessed by sedimentation at unit gravity (data not shown).

4F10 cells do not terminally differentiate

We asked if, after prolonged stimulation, 4F10 cells would terminally differentiate to the plasma cell state. 4F10 cells were stimulated with IL-5±IL-4 or LPS for 18 days. During this period, cells were recultured every 3 days with fresh cytokines or LPS. After each 3-day period, secreted IgA was assayed by ELISA and mIgA expression was determined by flow cytometry. As shown in Fig. 2a, the increase in IgA secretion by 4F10 cells, as detected after stimulation with LPS or IL-5±IL-4 for 3 days, was maintained over the 18-day period. Expression of mIgA decreased 3 days after stimulation with LPS or IL-5±IL-4 and remained decreased thereafter (Fig. 2b). Thus, even with prolonged stimulation, 4F10 cells did not lose membrane immunoglobulin expression, a property that would be expected if the cells had terminally differentiated.

Changes in doubling time of 4F10 cells were also characterized in these experiments over the 18-day culture period. As shown in Fig. 2c and Table 1, during the initial 3 days of culture there was a slight shortening of the doubling time of LPS- or IL-5±IL-4-stimulated cells. However, 6 or more days after beginning of stimulation doubling times of cytokine- or LPS-stimulated cells were markedly longer compared to controls. For example, after 12 days stimulation doubling times of

Table 2. IgA secretion of 4F10 cells in short-term cultures parallels cumulative IgA secretion in 3-day cultures*

Additions to culture	2-hr culture IgA ng/10^6 cells	2-hr culture Fold increase over unstimulated controls	3-day culture IgA ng/10^6 cells	3-day culture Fold increase over unstimulated controls
None	201±19	1·00±0·09	1357±93	1·00±0·07
LPS	2147±387	10·68±1·93	27,957±3674	20·60±2·71
IL-5	790±120	3·93±0·60	5107±914	3·76±0·67
TGFβ1	115±16	0·57±0·08	923±51	0·68±0·04

* 4F10 cells were cultured for 3 days at 2×10^4/ml in medium alone, or medium containing optimal concentrations of LPS, IL-5 or TGFβ1 (Table 1) after which cells were washed and recultured at 10^6/ml for 2 hr. The IgA concentration of culture supernatants was determined by ELISA. For comparison, IgA secretion, calculated as IgA concentration in supernatants divided by cell density, of the initial 3-day cultures are shown. Results are means±SEM of four independent experiments.

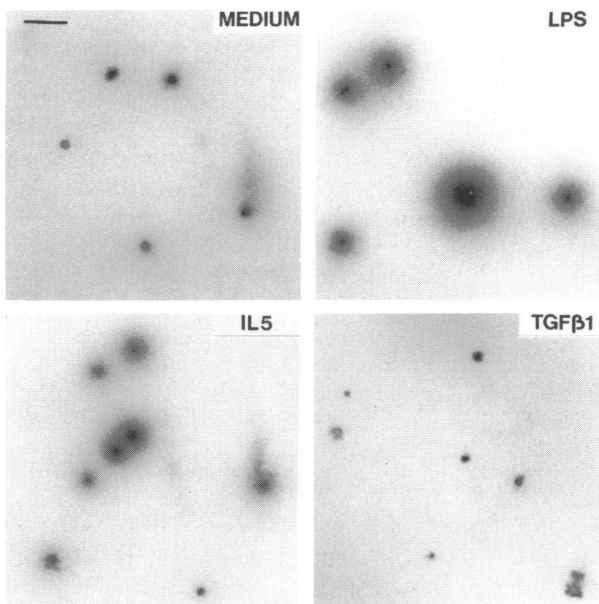

Figure 1. ELISPOT analysis of 4F10 cells. 4F10 cells were cultured for 3 days in medium alone or medium containing optimal concentrations of LPS, IL-5 or TGFβ1. Cells were analysed by ELISPOT assay as described previously.[8] Membranes from individual wells were removed and photographed at the same magnification. Bar in the top left corner represents 100 μm. As shown, spots formed by LPS-stimulated cells are three- to sixfold larger in diameter than spots of unstimulated cells. Spots from IL-5-stimulated cells were more heterogeneous in size and ranged from the spot size of control cells to fourfold the diameter of spots from control cells. Spots from TGFβ1-stimulated cells were equal or smaller than those from unstimulated cells.

Table 3. Effect of cytokines and LPS on membrane IgA expression of 4F10 cells*

Additions to culture	Membrane IgA expression†
None	1
LPS	0·446±0·076‡
IL-1β	0·899±0·041
IL-2	0·913±0·073
IL-4	1·007±0·013
IL-5	0·686±0·095‡
IL-6	0·961±0·098
IFN-γ	0·953±0·031
TGFβ1	1·061±0·053

* 4F10 cells cultured for 3 days with optimal concentrations of LPS or various cytokines were washed and stained with a FITC-labelled anti-IgA antibody, and then analysed for median fluorescence intensity (MFI) on a flow cytometer. Results are mean± SEM of three or more experiments.

† Membrane IgA expression = MFI of stimulated cells/MFI of unstimulated cells.

‡ Values significantly different from medium controls, $P < 0{\cdot}01$.

stimulated cells were between 16 and 36 hr while control cells showed a doubling time of 12 hr.

IgA secreted by 4F10 cells is mainly 7S monomeric IgA

IgA exists mainly as a monomer (7S IgA) in serum and as a dimer (10S IgA, or 11S IgA if complexed with secretory component) in mucosal secretions. As shown in Fig. 3, supernatants from 3-day cultures of unstimulated 4F10 cells contained 84% 7S monomeric IgA (fractions 9–14), and 16% polymeric IgA (fractions 15–23). Analysis of supernatants from cells stimulated with LPS or cytokines (IL-5, IL-2, TGFβ1, IL-5±IL-2, IL-5±IL-4) yielded a similar proportion of monomeric to polymeric IgA (data not shown). In contrast, the

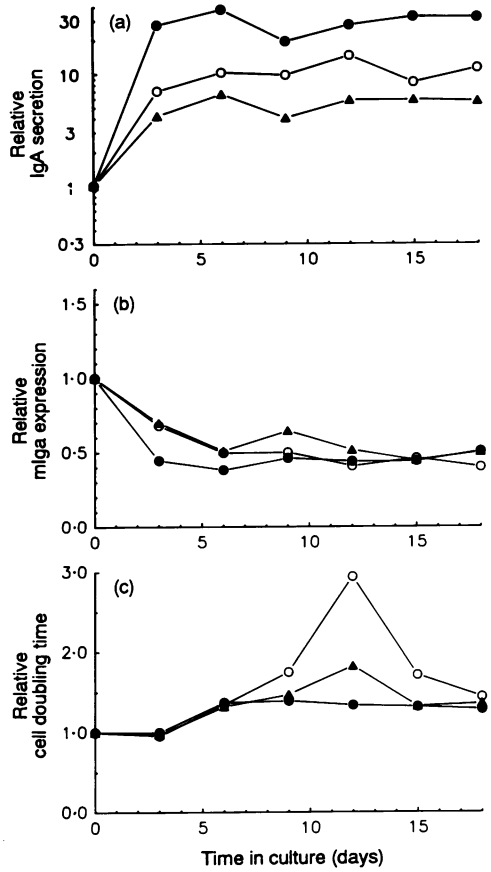

Figure 2. IgA secretion, membrane IgA expression and cell growth properties of 4F10 cells during prolonged stimulation. 4F10 cells (10^4 to 6×10^4/ml) were cultured in medium alone or medium containing optimal concentrations of LPS (●), IL-5 (▲), or IL-4 and IL-5 (○) for 18 days. During this period, cells were recultured every 3 days with fresh LPS or IL-5 ± IL-4. IgA secretion (a), membrane IgA expression (b) and cell doubling times (c) were determined at the end of each 3-day culture period. Data for stimulated cultures are presented as ratios relative to the values obtained in unstimulated cells. The cell doubling time of unstimulated cells was 12 hr.

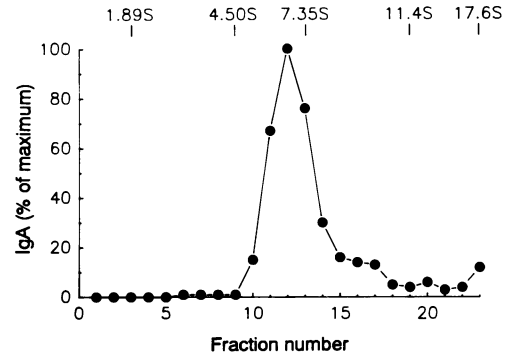

Figure 3. Size fractionation of IgA secreted by 4F10. Two-hundred microlitres of culture supernatant from unstimulated 4F10 cells were applied to a sucrose gradient and centrifuged as described in Materials and Methods. The gradient was fractionated and analysed for the presence of α heavy chains. Monomeric IgA (7S) peaks in fraction 12, and dimeric IgA (10S) peaks in fraction 17. α chain concentration in each fraction is given as a percentage of the maximum concentration found in the peak fraction. Standards include a mixture of proteins with known sedimentation coefficients and are indicated by arrows. Ferritin (17.6 S) was found in the pellet (fraction 23).

parental, IgM expressing, cell line CH12.LX[15] secreted more than 80% of the IgM in the polymeric form (data not shown).

Increased IgA secretion is paralleled by increased mRNA levels for secreted IgA and for J chain

To gain insight into the molecular mechanisms underlying increased IgA secretion after cytokine or LPS stimulation, Northern blot analysis of total cellular RNA from unstimulated and stimulated 4F10 cells was performed (Fig. 4). Hybridization with a cDNA probe specific for the α constant region (Fig. 4a), which detects mRNA for both membrane and secreted IgA, revealed three bands of 3·0, 2·0 and 1·6 kb. The two slow migrating bands represent mRNA for membrane IgA (α_M mRNA), while the 1·6 kb band represents mRNA for secreted IgA (α_s mRNA) (data not shown).[23,24] In unstimulated 4F10 cells the level of α_s mRNA approximately equalled the sum of the two α_M mRNA. In contrast, after stimulation with LPS for 3 days the level of α_s mRNA was increased sevenfold, while the level of 3·0 kb α_M mRNA was 30% decreased and the level of 2·0 kb α_M mRNA was essentially unchanged. Moreover, after stimulation with IL-5, or IL-4 and IL-5 for 3 days, the levels of α_s mRNA were four- or sevenfold increased, respectively, whereas the levels of 3·0 kb α_M mRNA and 2·0 kb α_m mRNA were not significantly different from controls. Hybridization with a probe specific for the immunoglobulin κ light chain constant region (κ) yielded a single band of 1·1 kb. After stimulation with LPS the level of κ mRNA was fourfold increased, and two- or fourfold increased after stimulation with IL-5 or IL-4 and IL-5, respectively. None of the other cytokines tested (IL-1, IL-2, IL-4, IL-6, IFN-γ, TGFβ1) resulted in mRNA levels of α_s, α_M or κ significantly different from controls (Fig. 4a). Thus, increased IgA secretion was reflected by a proportional increase in α_s and κ mRNA levels.

To determine if the predominantly monomeric nature of the IgA secreted by 4F10 cells is caused by a lack of J-chain expression, steady-state levels of J-chain mRNA were also investigated by Northern blot analysis (Fig. 4b). Hybridization with a cDNA probe specific for J chain revealed the expected[25] major band of 1·4 kb, and two less intense bands of 2·3 and 1·1 kb. Unstimulated 4F10 cells contained low levels of J-chain mRNA. In contrast, after stimulation with LPS, IL-4 ad IL-5, or IL-5 alone, J chain mRNA levels were increased 16-fold, eightfold and threefold, respectively.

DISCUSSION

The murine IgA-expressing B-cell lymphoma CH12.LX. C4.4F10 was used to define the effect of cytokines on IgA secretion, membrane IgA expression, IgA polymerization and IgA B-cell growth. Each cytokine studied had previously been reported to alter IgA production by heterogeneous populations of freshly isolated murine B cells.[1-13] As reported here, cytokine effects of IgA secretion at the single-cell level were distinguished from those on cell growth and survival by employing ELISPOT analysis to assess the proportion of IgA-secreting cells, short-term cultures to determine the secretory state of the cells, and measurements of cell growth and viability. These distinctions

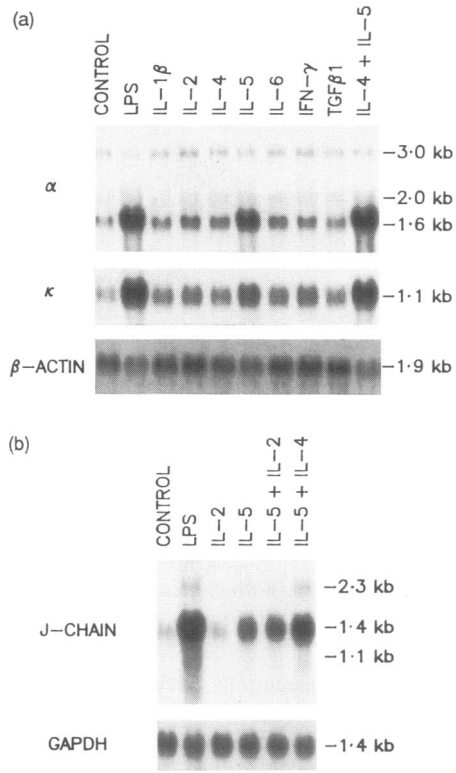

Figure 4. Northern blot analysis of immunoglobulin heavy and light chains and J chain in 4F10 cells. 4F10 cells were cultured for 3 days with optimal concentrations of LPS or various cytokines as indicated in the figure, and RNA was extracted. (a) Ten micrograms total RNA was size-fractionated on an agarose/formaldehyde gel and blotted onto nitrocellulose. Blots were hybridized with ^{32}P-labelled probes for α heavy chain, κ light chain and β actin as indicated in the figure. Sizes of mRNA were derived from comparison with RNA molecular weight markers run in parallel. (b) Forty micrograms total cellular RNA was size-fractionated on an agarose/formaldehyde gel, transferred to a nitrocellulose membrane and hybridized with ^{32}P-labelled probes for J chain and, subsequently, GAPDH.

are important, but taken together, have not been reported before in studies of cytokine-stimulated B cells. For example, TGFβ1 increases switching of membrane IgM-bearing B cells to IgA expression.[1,4] However, as demonstrated here, TGFβ1 actually inhibits IgA secretion by B cells already committed to IgA production. Notably, the inhibitory effect of TGFβ1 on IgA secretion was independent of its inhibitory effect on cell growth. Earlier studies of TGFβ1 effects on the IgA response measured cumulative IgA secretion in B-cell cultures that were co-stimulated with LPS.[1-3] However, as we have shown, stimulation of 4F10 cells with LPS can completely reverse the inhibition of IgA secretion induced by TGFβ1 alone. Thus, at the cellular level, TGFβ1 appears to increase switching of IgM B cells to expression of the IgA class, while inhibiting IgA secretion and B-cell proliferation.

IL-5 increased IgA secretion by a large fraction of 4F10 cells. This is consistent with previous observations that IL-5 increases cumulative IgA secretion among heterogeneous populations of freshly isolated membrane IgA-bearing B cells[5-9] or B-lymphoma cells[10] in culture. We and others previously reported that IL-4 co-operates with IL-5 to synergistically increase cumulative IgA secretion in cultures of freshly isolated B cells.[5-8] Using 4F10 cells, we confirmed the co-operative activity of IL-4 and IL-5 on IgA secretion, and demonstrated that increased IgA secretion can be explained by an increase in the amount of IgA secreted per cell. In contrast, co-stimulation of 4F10 cells with IL-5 and IFN-γ inhibited the IL-5-stimulated increase in IgA secretion, although IFN-γ alone did not significantly alter IgA secretion. In this regard, IFN-γ also inhibited the IL-5-mediated increase in IgA secretion among freshly isolated, LPS-stimulated murine spleen B cells.[5] In contrast, IFN-γ synergized with IL-5 to increase antigen-specific IgA responses[13] indicating IFN-γ may have different effects on IgA B cells depending on their state of activation and development.

Unstimulated as well as cytokine- or LPS-stimulated 4F10 cells secrete a constant ratio of monomeric to polymeric IgA. Thus, IgA polymerization appears to be regulated co-ordinately with IgA secretion in this cell line, a notion consistent with levels of increased J-chain mRNA after cytokine or LPS stimulation. Nonetheless, the role of J chain in regulating IgA polymerization is not certain. In this regard, J-chain expression has been reported to be limiting for the secretion of polymeric IgM, suggesting an important function for J chain in IgM polymerization.[26] However, others have found high levels of J-chain expression without a corresponding increase in polymeric IgM secretion.[27] Expression of an exogenously introduced J-chain gene in 4F10 cells may help to define more exactly the role of J chain in IgA polymerization.

IL-5 and LPS initially stimulated and later inhibited the proliferation of 4F10 cells. Stimulatory effects of IL-5 and LPS on B-cell proliferation have been shown before in short-term cultures of freshly isolated B cells[28] and for IgM-expressing B-cell lines.[29-31] However, the inhibitory effects of these agonists on B-cell proliferation after more prolonged stimulation have not been reported previously. Inverse relationships between differentiation and cell growth are generally recognized for differentiating systems including gut and skin epithelium, haemopoiesis and some aspects of lymphopoiesis, as well as for tumour cell lines[32] and decreased proliferation is an integral part of normal B-cell differentiation (i.e. plasma cells do not proliferate and are short-lived). Thus, the observed cytokine-induced growth inhibition of 4F10 cells, which is paralleled by increased IgA secretion and decreased membrane IgA expression, appears to be part of a differentiation programme that can be induced in 4F10 cells either by IL-5, IL-4 and IL-5, or LPS. Moreover, cytokine-induced growth inhibition of 4F10 cells suggests a potential treatment strategy for B-cell lymphomas.

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