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Regulation of cytokine gene expression by adjuvants *in vivo*

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SUMMARY

Antibody isotype affects biological activity of the antibodies and therefore should be considered in prevention of disease by vaccination. In previous reports, we demonstrated that adjuvants affect the antibody isotype switching process and favour the production of certain isotypes. The present study extends these findings and shows fundamental differences in the cytokine induction pattern according to the adjuvant used. Cytokine mRNA levels were determined by *in situ* RNA–RNA hybridization performed on splenocytes isolated from mice injected with different adjuvants. The results revealed that Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA), $Al(OH)_3$ and QuilA administration results in a type-2 (humoral) response, increasing IL-4, IL-5 and IL-13 gene expression, while poly I:C exhibits a type-1 (cell-mediated) response, increasing the production of interferon-gamma (IFN-γ), IL-2 and IL-6 mRNA. Finally, $BeSO_4$ and poly A:U augment IL-5 and IL-6 mRNA production, while lipopolysaccharide (LPS) and LiCl augment IL-6 and tumour necrosis factor-alpha (TNF-α) mRNA production. Also, the adjuvants appear capable of overcoming the inherent IL-2/IFN-γ and IL-4 dichotomy of C57Bl/6 and BALB/c mice, respectively, in response to cellular antigens such as Leishmania and herpessimplex virus (HSV). The overall data suggest that adjuvants direct the isotype switching process via induction of certain cytokines, a finding that can be useful in selection of the most efficient isotype of protective antibodies for disease prevention by vaccination.

Keywords adjuvant cytokine vaccine isotype switching *in situ* hybridization

INTRODUCTION

Vaccination is still considered to be the most effective way to prevent infectious diseases [1,2]. Modern biochemical, biosynthetic and recombinant techniques have permitted the production of increasingly pure antigens to induce specific immune responses. However, often these antigens are only weak immunogens, and for this reason adjuvants are being used to increase their potency. An adjuvant acts upon the humoral response by increasing the quantity and the affinity and by modulating the isotype of the induced antibodies [3]. Antibody isotype affects crucial features such as tissue distribution, complement activation, binding to high-affinity receptors on monocytes and other cell types. It has been recently established that immunoglobulin class switching is mainly regulated by selective activation of specific T cell subsets, characterized as Th1 and Th2 [4]. These two subsets are distinguished by the cytokines they secrete. The Th1 subset produces IL-2, interferon-gamma (IFN-γ) and tumour necrosis factor-beta (TNF-β), whereas the Th2 subset produces IL-4 and IL-5 [5,6]. The differences in cytokine profile is thought to reflect different biological functions of these two subsets. Whereas the Th1 subset is responsible for classical cell-mediated immunity, the Th2 subset functions more as a helper for B cell activation.

In previous studies we have shown that antibody isotype selection is affected by the adjuvant used for immunization or for *in vitro* stimulation [7–9]. The purpose of the present study was to investigate the effect of certain adjuvants on the cytokine induction pattern produced in response to a thymus-dependent (TD) antigen. *In situ* RNA–RNA hybridizations were performed to evaluate the cytokine mRNA-producing spleen cells, while cytokine protein-producing cells were evaluated by a immunocytochemical method. Our results demonstrate that different adjuvants elicit different cytokine profiles, suggesting that adjuvants direct the antibody isotype switching process via the induction of certain cytokines.

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MATERIALS AND METHODS

Animals

Three- to five-month-old inbred BALB/c or C57Bl/6 mice of either sex were used in this study. The animals, which had been bred in

our laboratory, were housed under standard conditions and received a diet of commercial food pellets and water *ad libitum*.

Antigen and adjuvants

Freund's complete adjuvant (FCA) and Freund's incomplete adjuvant (FIA) were purchased from Difco (Detroit, MI). Lipopolysaccharide (LPS) from *Escherichia coli* 055:B5 was kindly provided by the Department of Immunology of Karolinska Institute (Stockholm, Sweden). $AlSO_4$, $BeSO_4$, poly I, C, U, A were purchased from Sigma (St Louis, MO). FITC-labelled human gammaglobulin (HGG) as well as adjuvants were prepared as previously described [7].

Immunizations

Groups of three mice were immunized intraperitoneally with 150 μg of antigen (HGG–FITC) in the presence or absence of different adjuvants. The amount of adjuvant injected per mouse was as follows: FCA 0·1 ml, LPS 100 μg, $Al(OH)_3$ 20 μg, LiCl 100 μg, $BeSO_4$ 50 μg, QuilA 100 μg and poly I:C or A:U 1·2 mg [7].

Fixation of spleen cells for in situ *hybridization and immunocytochemistry*

Spleen cells obtained at different times after immunization were fixed on glass microscope slides using a cytocentrifuge (1×10^5 cells/slide in Hank's balanced salt solution (HBSS) with 10% fetal calf serum (FCS)). Slides were air-dried for 5 min and fixed for 1 min in 4% paraformaldehyde in BSS with subsequent storage in 70% ethanol at 4°C.

Probe preparation

^{35}S-UTP-labelled sense and anti-sense RNA probes were prepared by *in vitro* transcription using SP6, T3 or T7 RNA polymerase (New England Biolabs, Beverly, MA) and a linearized plasmid containing the appropriate cytokine cDNA. mIL-4 and mIL-5 cDNAs were kindly donated by Dr Paschalis Sideras (University of Umea, Sweden), mTNF-α cDNA by Dr Bruce Beutler (University of Texas Southwestern Medical Center, Dallas, TX) and mIL-13 cDNA by Dr R. L. Coffman (Department of Immunology, DNAX Research Institute, Palo Alto, CA). mIL-2 and mIFN-γ cDNAs were purchased from LMBP Culture Collection (Gent, Belgium). mIL-1 and mIL-6 cDNAs are identical to those we previously described [10].

In situ *hybridization*

In situ hybridization was performed as previously described [10]. Paraformaldehyde-fixed spleen cells were rinsed twice in 2×SSC and acetylated for 10 min in 0·1 M triethanolamine pH 8·0 containing 0·25% acetic anhydride. After acetylation, the slides were washed once in 2×SSC and PBS and transferred in 0·1 M glycine, 0·1 M Tris–HCl pH 7·0 for 30 min. Following two additional washed in 2×SSC, slides were placed in 50% formamide, 2×SSC at 55°C for 5 min. Hybridization (20 μl of probe mixture: 50% formamide, 10% dextran sulphate, 1 mg/ml *E. coli* tRNA, 1 mg/ml sheared salmon sperm DNA, 2 mg/ml bovine serum albumin (BSA), 10 mM DTT, ^{35}S-labelled RNA probe of 1×10^6 ct/min per slide, in 2×SSC) was carried out at 50°C for 12 h. After hybridization, slides were washed in three changes of 50% formamide, 2×SSC at 52°C, rinsed well in 2×SSC and treated with RNAse A for 30 min at 37°C. Afterwards, slides were rinsed in 2×SSC, washed with 50% formamide, 2×SSC at 52°C, and finally rinsed well before being dehydrated sequentially in 70%, 80% and 90% ethanol. Hybridized slides were dipped in Kodak NTB2 nuclear track emulsion (Easterman Kodak Co., Rochester, NY), developed in Kodak dental x-ray fixer. The slides were counterstained with Giemsa solution and air dried. Five thousand cells from each sample were counted and hybridization density was estimated by classifying the positive cells according to the number of grains overlying the cell. The results were also represented as the percentage of positive cells. Positive cells were classified as those exhibiting more than five autoradiographic grains.

Cell cultures and antibody purification

11B11 and XMG1.2 hybridoma cell lines producing rat IgG anti-mouse-IL-4 and anti-mouse-IFN-γ antibodies, respectively, were generously provided by Dr R. L. Coffman. Cell lines were cultured in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 50 U/ml penicillin, 50 mg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol (2-ME) and geneticin G-418 sulphate at 37°C. Two days before supernatant recovery, cells were cultured in the absence of geneticin G-418 sulphate. Antibodies were purified by ammonium sulphate precipitation and ion exchange chromatography on DEAE-matrix as previously described [11].

Immunocytochemistry

Cytokine-secreting spleen cells were measured by a slight modification of an immunocytochemical method [12]. Paraformaldehyde-fixed cell preparations were rehydrated in 70%, 50% and 30% ethanol. Endogenous peroxidase was inhibited by dipping the slides in methanol containing 0·1% hydrogen peroxide for 20 min. After blocking with 5% normal rabbit serum for 30 min at 37°C, slides were incubated with rat IgG anti-mouse-IL-4 or rat IgG anti-mouse-IFN-γ for 60 min at 37°C and washed extensively with PBS. Afterwards, slides were incubated with rabbit anti-rat-IgG conjugated with peroxidase (Sigma) for 60 min at 37°C and washed with PBS. Thirty microlitres of 0·5 mg/ml *o*-dianiside (Sigma) in PBS containing 0·01% hydrogen peroxide were added for 30 min. Following extensive washing with PBS, slides were counterstained with Giemsa solution.

ELISA-SPOT

Specific antibody-secreting cells were enumerated by a modification of the ELISA-SPOT test, as previously described [13]. Briefly, 100 μl of 50 μg/ml HGG–FITC in carbonate-bicarbonate buffer pH 9·6 were added in 96-well flat-bottomed microtitre plates and left overnight at 4°C. The antigen-coated wells washed with PBS and incubated for 30 min at 37°C with RPMI 1640 containing 1% BSA. Spleen cells (1×10^6) obtained 7 days post-immunization were added to coated wells in two-fold serial dilutions. The plates were subsequently incubated for 3 h at 37°C. Wells were washed with PBS and incubated with 100 μl alkaline phosphate (AP)-conjugated goat anti-mouse IgG1 or IgG2a antibodies (ICN Biochemicals Inc, Costa Mesa, CA) for 2 h at 37°C. After extensive washing with PBS, 0·05% Tween 20, 100 μl of AP substrate 5-bromo-4-chloro-3-indolylphosphate (1 mg/ml; Sigma) in 1 M methyl-1-propanol buffer containing 5 mM $MgCl_2$, 0·01% Triton X-405, 0·01% NaN_3 0·6% 36°C gelling agarose were added to each well. Plates were incubated overnight at 37°C. Spots were counted microscopically and results were expressed as the number of antibody-secreting cells per spleen.

RESULTS

In order to investigate the effect of various adjuvants on cytokine mRNA production by spleen cells, BALB/c mice were either immunized with antigen (FITC–HGG), adjuvant, or with both. The number of cytokine mRNA-producing cells and the relative amount of cytokine mRNA per cell were determined from day 1 to day 5 post-treatment. Figure 1 shows typical examples of hybridized spleen cells using either sense or antisense probes. Untreated mice showed no cytokine mRNA production, while treatment with antigen alone resulted in a relatively low induction of most of the cytokines tested. However, when adjuvant was added, a dramatic change of the cytokine mRNA induction profile was observed.

Induction of IL-4 mRNA

The adjuvants FCA, FIA, $Al(OH)_3$ and QuilA all substantially increase the frequencies of IL-4 mRNA-producing cells (Fig. 2a), as well as the number of autoradiographic grains per positive cell (Table 1). Kinetics of IL-4 mRNA production indicated that maximum levels were observed by day 3 post-injection. More or less the same levels of induction were observed when FCA, FIA and QuilA were injected in the presence of antigen. $Al(OH)_3$ poses an exception to the above. When this adjuvant was administered in combination with antigen, higher levels of IL-4 mRNA-producing cells were detected, suggesting a synergistic effect on IL-4 gene induction (Fig. 2a).

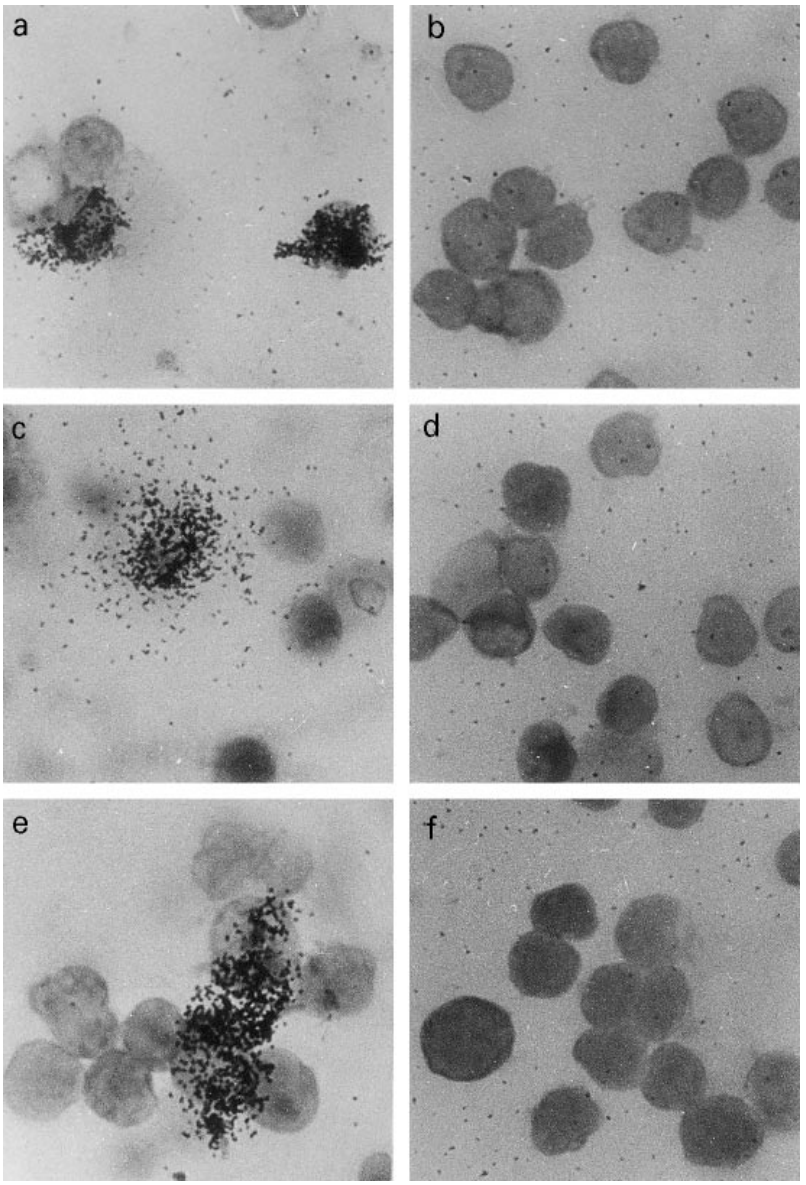

Fig. 1. *In situ* hybridization for the determination of cytokine mRNA-producing spleen cells after immunization with adjuvants. mIL-4 antisense (a) and sense (b) RNA probe on cell treated with Freund's complete adjuvant (FCA) + human gammaglobulin (HGG)–FITC, mIFN-γ antisense (c) and sense (d) RNA probe on cells treated with poly I:C + HGG–FITC, and mIL-6 antisense (e) and sense (f) RNA probe on cells treated with lipopolysaccharide (LPS) + HGG-FITC.

Induction of IL-13 and IL-5 mRNA

Administration of FCA, FIA, $Al(OH)_3$ or QuilA, resulted in an increased number of IL-13 and IL-5 mRNA-producing cells (Fig. 2b,c) as well as an increase in autoradiographic grains per positive cells (Table 1). $Al(OH)_3$ presents an exception by augmenting mRNA induction when acting together with antigen. However, in the case of IL-5 two additional adjuvants, $BeSO_4$ and poly A:U, were capable of inducing this cytokine's mRNA. Maximum expression was observed by day 3 post-injection for both the above cytokines.

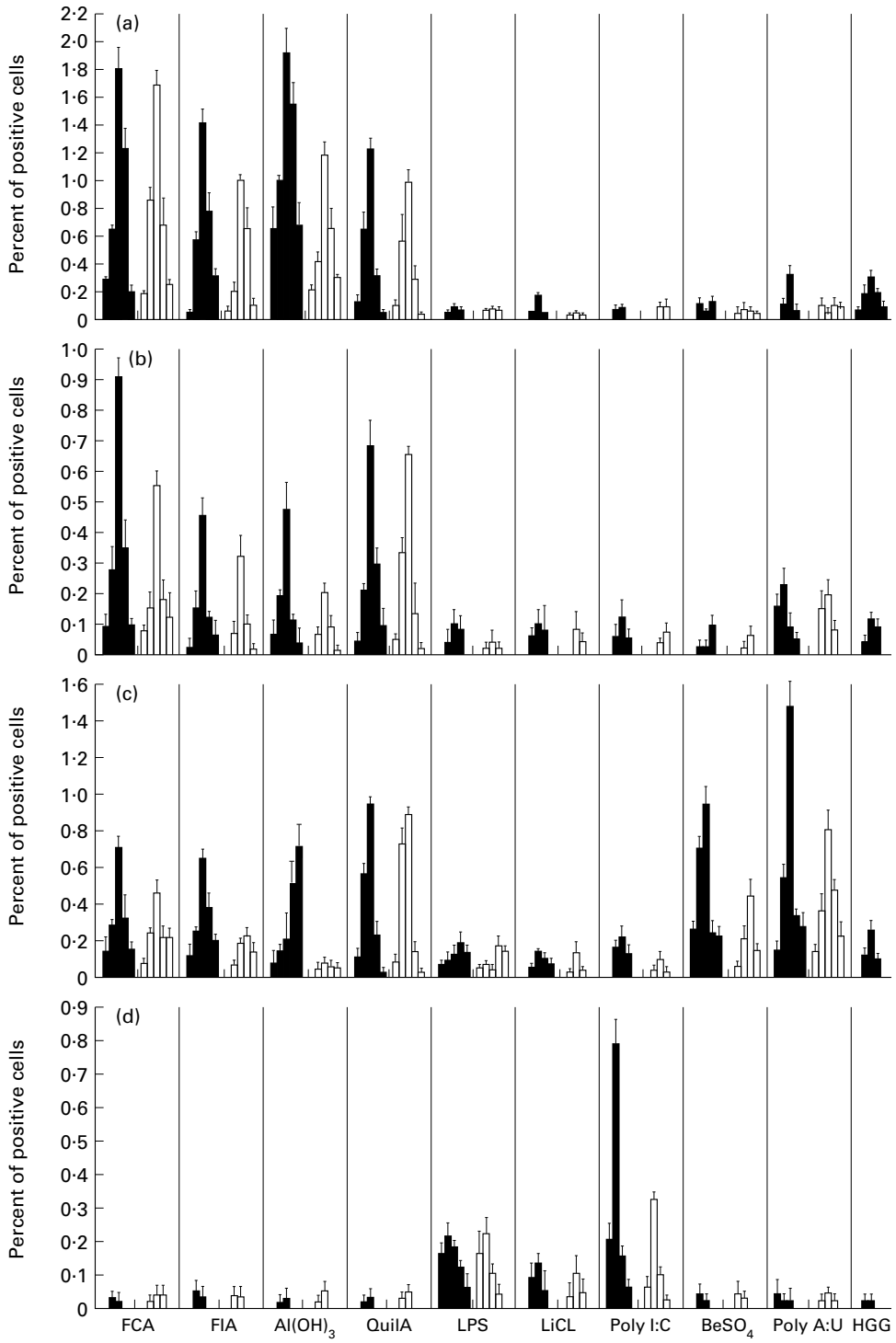

Fig. 2. Kinetics of cytokine mRNA-producing spleen cells after immunization of BALB/c mice with various adjuvants in the presence (■) or in the absence (□) of antigen. Spleen cells were harvested on days 1, 2, 3, 4 and 5 post-injection and *in situ* hybridization was performed using the following probes: (a) mIL-4; (b) mIL-13; (c) mIL-5 and (d) mIFN-γ. Each bar represents one time point. Results are expressed as mean ± s.e.m. of three independent experiments with three animals in each group. FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; LPS, lipopolysaccharide; HGG, human gammaglobulin.

Table 1. Relative cytokine mRNA production in splenocytes of adjuvant-injected BALB/c mice

	IL-4	IL-13	IL-5	IFN-γ	IL-2	IL-6	TNF-α	IL-1
FCA	++++	++++	++	−	++	+++	−	+++
FCA+Ag	++++	++++	++	−	++	+++	++	+++
FIA	+++	++	+	−	+	+++	−	++
FIA+Ag	+++	+++	++	−	++	+++	++	++
$Al(OH)_3$	+++	++	+	−	++	+	−	+
$Al(OH)_3$+Ag	++++	+++	++	−	++	+	−	+
QuilA	+++	+++	+++	−	++	−	−	++
QuilA+Ag	+++	+++	+++	−	++	−	−	++
LPS	−	−	−	++	+	++++	++++	++++
LPS+Ag	−	−	−	++	+	++++	++++	++++
LiCl	−	−	−	+	++	++	−	+
LiCl+Ag	−	−	−	+	++	++	−	+
Poly I:C	−	−	−	++	+++	+++	−	++
Poly I:C+Ag	−	−	+	++++	++++	+++	−	++
$BeSO_4$	−	−	++	−	++	+++	−	+
$BeSO_4$+Ag	+	−	+++	−	++	++++	−	+
Poly A:U	−	+	+++	−	+++	++++	−	++
Poly A:U+Ag	+	+	++++	−	+++	++++	+	++
Ag	+	+	+	−	+	+	−	+

The relative levels of production were estimated according to the density of hybridization (number of autoradiographic grains per positive cell).

−, No induction was observed; ++++, the maximum of induction observed for the particular cytokine; Ag, the antigen used (HGG–FITC).

FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; LPS, lipopolysaccharide.

Induction of IFN-γ and IL-2 mRNA

The induction profile of IFN-γ mRNA by the adjuvants examined was radically different. None of the adjuvants FCA, FIA, $Al(OH)_3$, $BeSO_4$ and poly A:U induced IFN-γ mRNA, while positive cells were found only after immunization with LPS, LiCl and poly I:C (Fig. 2d). However, in the case of LPS and LiCl, the induction level was significantly lower when compared with the other cytokines examined. The stronger IFN-γ inducer was found to be the adjuvant poly I:C, giving maximum mRNA production by day 2 post-treatment. All the adjuvants tested increased IL-2 mRNA production. Poly I:C was also the stronger IL-2 mRNA inducer (Fig. 3a).

Induction of IL-6, TNF-α and IL-1 mRNA

FCA, FIA, LPS, poly I:C, $BeSO_4$ and poly A:U were found to induce IL-6 mRNA (Fig. 3b). LPS was the strongest IL-6 mRNA inducer. Maximum response was observed by day 3–4 after induction with LPS, $BeSO_4$, poly I:C and poly A:U, and with adjuvants FCA and FIA by day 1. The strongest inducer for TNF-α mRNA was also LPS, although in this case the optimal response was delayed, in comparison with other cytokines, until day 4 post-treatment (Fig. 3c). FCA, FIA and LiCl, although having almost no effect was administered alone, induced TNF-α mRNA in the presence of antigen. None of the other adjuvants tested was an inducer of TNF-α mRNA. In the case of IL-1, almost all the adjuvants tested caused mRNA induction, with a maximum by day 1, except for $Al(OH)_3$ and LiCl (Fig. 3d).

All the above results are presented in Table 1 as the relative levels of gene expression according to the hybridization density (the number of autoradiographic grains per positive cell).

IL-4 and IFN-γ protein production after immunization with FCA and poly I:C

BALB/c mice were injected intraperitoneally with antigen in the presence of FCA or poly I:C. Spleen cells obtained from 30 min to 3 days after immunization were examined for IL-4- and INF-γ-secreting cells by immunocytochemistry. Both IL-4 and IFN-γ displayed an early and a late production in response to FCA and poly I:C, respectively (Fig. 4).

Induction of IL-4 and IFN-γ in BALB/c and C57Bl/6 mice

Spleen cells derived from BALB/c and C57Bl/6 mice, immunized against antigen in the presence or absence of FCA or poly I:C, were examined for IgG1 and IgG2a antibodies by day 7, as well as IL-4 and IFN-γ production by day 2. Although the percentage of antibody-producing cells was higher in BALB/c than in C57Bl/6, no differences were observed in dominant isotype or cytokine secreted (Fig. 5). In both stains of mice, FCA increased the production of IL-4 with subsequent IgG1 secretion, while poly I:C increased the production of IFN-γ with subsequent IgG2a secretion (Fig. 5).

DISCUSSION

Adjuvants have been used as a traditional approach to overcome the problem of weak immunogenicity during development of modern vaccines. A number of separate compounds have been used as adjuvants, presenting large variety of conformations and actions via different pathways [3]. We have previously found that treatment with different adjuvants results in secretion of antibodies of different isotype [7,8]. Isotype selection is a process known to be controlled by certain cytokines, which act either as switch or

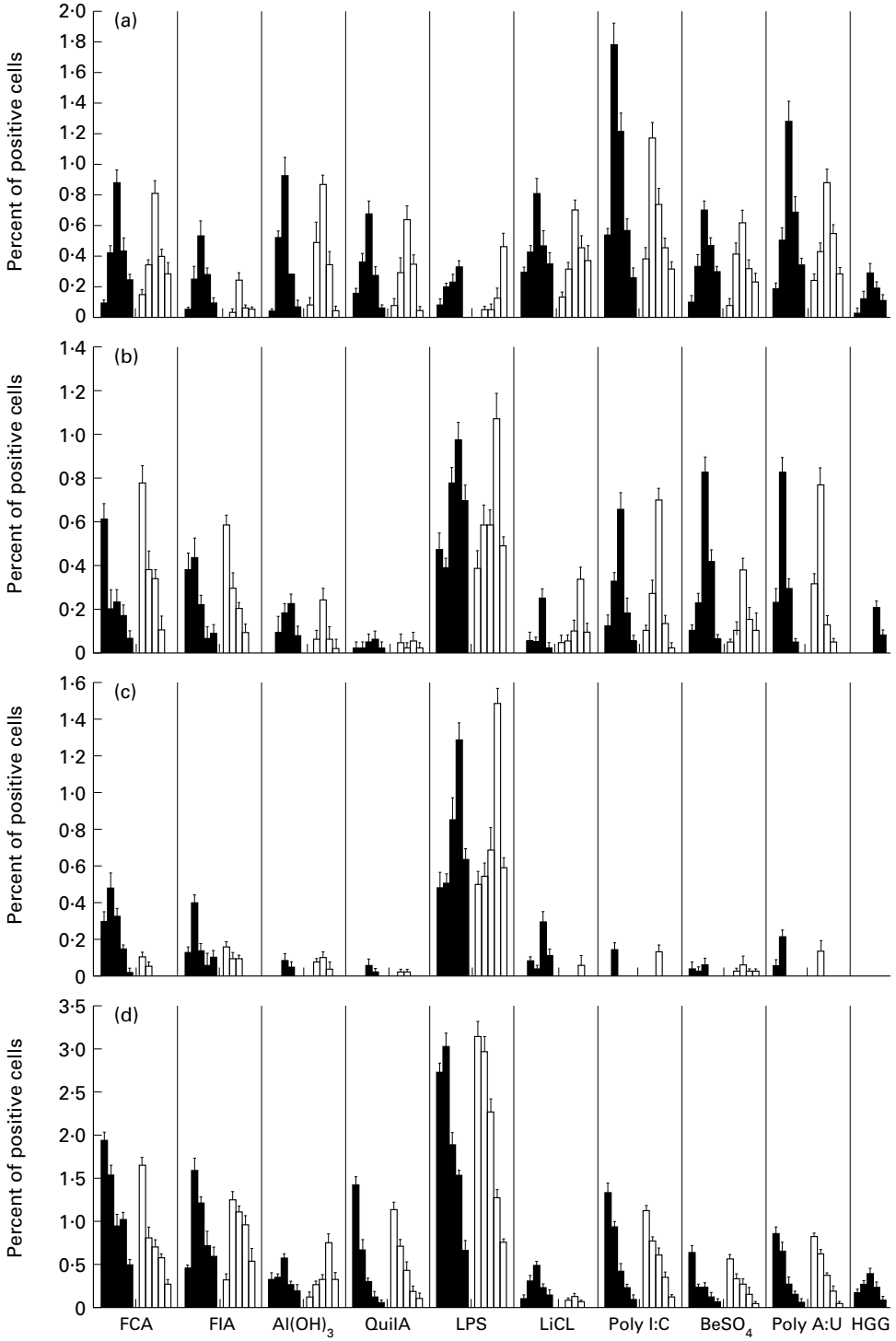

Fig. 3. Kinetics of cytokine mRNA-producing spleen cells after immunization of BALB/c mice with various adjuvants in the presence (■) or in the absence (□) of antigen. Spleen cells were harvested on days 1–5 post-injection and *in situ* hybridization was performed using the following probes: (a) mIL-2, (b) mIL-6, (c) mTNF-α, and (d) mIL-1. Each bar represents one time point. Results are expressed as mean ± s.e.m. of three independent experiments with three animals in each group. FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; LPS, lipopolysaccharide; HGG, human gammaglobulin.

post-switch differentiation factors [14–16]. Employing the same experimental model, we now demonstrate that different adjuvants also vary in their ability to induce synthesis of certain cytokine mRNAs in murine spleen cells. In addition, our data support previous studies suggesting that adjuvants modulate the isotype selection process via induction of certain cytokines.

IL-4 and IL-13 mRNAs were induced by FCA, FIA, $Al(OH)_3$ and QuilA. It is well established that these lymphokines are

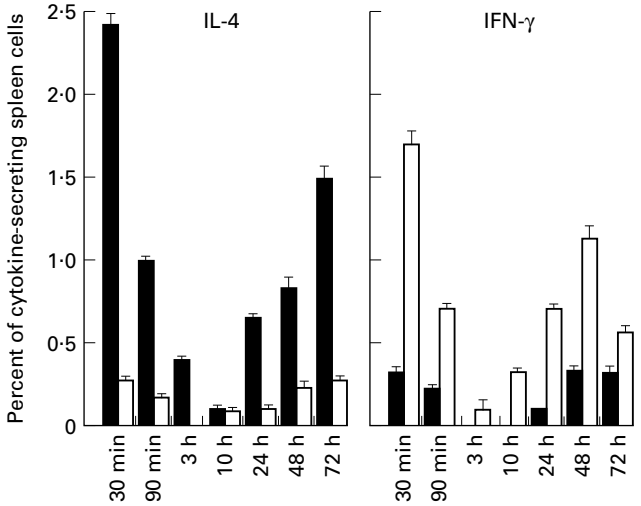

Fig. 4. Kinetics of cytokine-secreting spleen cells after immunization of BALB/c mice with human gammaglobulin (HGG)–FITC in the presence of Freunds' complete adjuvant (FCA) (■) or poly I:C (□). Spleen cells were harvested at 30 min to 72 h post-injection and IL-4 and IFN-γ protein production was demonstrated by immunocytochemistry. Results are expressed as mean ± s.e.m. of three independent experiments with three animals in each group.

produced by the Th2 subset, one of the $CD4^+$ T cell subpopulations that control the major features of specific immune response. In addition, IL-4 and IL-13 are known to act as switching factors, determining the production of IgG1 and IgE antibodies [17–21]. In previous studies we have shown that treatment with FCA, FIA, $Al(OH)_3$ and QuilA results in IgG1 production. Therefore, the present study strongly suggests that the above four adjuvants promote IgG1 production via Th2 stimulation and IL-4 and IL-13 induction.

IL-5 is a Th2-derived lymphokine that has been found to stimulate antibody production by enhancing both proliferation and differentiation of B-cells. IL-5 is reported to co-operate with IL-4 in IgG1 and IgE induction as a 'post-switch' B cell differentiation factor, acting upon mIgG1- and mIgE-bearing B cells, but not mIgG1- or mIgE-negative B cells, and resulting in their differentiation into antibody-secreting cells [22,23]. Therefore, our present data showing that IL-5 mRNA synthesis is induced by FCA, FIA, Al(OH)3 and QuilA suggest that the enhanced production of IgG1 isotype caused by the above adjuvants is due to IL-5 'post-switch' differentiation action. $BeSO_4$ and poly A:U, which were previously found to augment IgG2b production [7], also increase IL-5 mRNA levels, suggesting that IL-5 may be implicated in IgG2b production.

We have previously shown that administration of poly I:C results in an IgG2 response [7]. IgG2a production is known to be controlled by IFN-γ, which acts as an IgG2a switch factor [24,25]. Therefore, our present finding that poly I:C increases IFN-γ mRNA induction strongly suggests that poly I:C augments IgG2a production via Th1 activation and IFN-γ production. Our present data show that all adjuvants increase the production of IL-2, suggesting that IL-2 exerts no selective effects on the production of a particular isotype.

Almost all the adjuvants tested, with the exception of $Al(OH)_3$ and LiCl, enhanced IL-1 mRNA expression with a maximum by day 1 post-injection. This observation is consistent with the fact that IL-1 plays a crucial role in the initiation of the immune response [26,27].

IL-6 is another cytokine involved in the initiation of the immune response, acting synergistically with IL-1 [28–30]. The increased IL-6 mRNA production mediated by LPS, FCA and FIA by day 1 suggests that these adjuvants establish an immune response via IL-6 release. Recent studies have indicated that IL-6 also promotes the differentiation of B cells [31–33]. In our experiments, kinetics reveal that poly A:U and $BeSO_4$ administration results in IL-6 mRNA production by day 3, a profile similar to the induction of IL-5 after administration of the same adjuvants. Poly A:U and $BeSO_4$ have been previously shown to increase IgG2b secretion [7]. This co-induction suggests that IL-6 may be implicated in IgG2b production, acting synergistically with IL-5. Furthermore, IL-6 mRNA production is enhanced by day 3 after poly I:C treatment. Since poly I:C enhances IgG2a [7] and INF-γ production, then it is possible that IL-6 acts upon IFN-γ-switched IgG2a B cells, causing their differentiation into plasma cells. LPS and LiCl treatment were previously found to enhance IgG3 production [7]. The fact that LPS and LiCl are IL-6 and TNF-α mRNA inducers by day 4 leads to the assumption of a possible synergistic action of IL-6 and TNF-α on IgG3 production. In conclusion, our results suggest that IL-6 acts as a 'post-switch' B cell differentiation factor and promotes IgG2a, IgG2b and IgG3 antibody production, depending on other cytokines induced.

In the present study, an early (30 min) and a late (3 day) IL-4 and IFN-γ production was observed in response to FCA and poly I:C, respectively. Other investigators have demonstrated that early IL-4 secretion is essential for naive T cell differentiation into Th2, while early IFN-γ production is critical for naive T cell differentiation into Th1 [34]. Since in a primary response antigen-specific T cells are present at a very low frequency, the same investigators have suggested that other cells can influence T cell differentiation by secreting these cytokines. IFN-γ early production may be contributed by natural killer (NK) cells, while IL-4 may derive from mast cells and basophils [34]. Following T cell differentiation, FCA and poly I:C, as our results suggest, direct isotype selection via a late (Th1- or Th2-derived) IL-4 and IFN-γ production. However, studies were underway to characterize these early-stage cytokine-producing cells.

In order to examine the possibility of strain-specific influences on the cytokine production pattern and therefore the isotype produced, two different strains of mice were used. BALB/c mice are susceptible to infection by cellular pathogens such as Leishmania, indicating a weak cell-mediated immune response; they produce mainly IL-4 and other Th2-derived cytokines leading to a strong humoral immune response. C57Bl/6 mice are resistant to cellular pathogens and exert an effective cell-mediated immune response, producing IL-2 and IFN-γ [35–38]. Although BALB/c and C57Bl/6 have very different immune response dominance, our results show that FCA increases IL-4 and IgG1 production, while poly I:C increases IFN-γ and IgG2a production in both strains. These results suggest that the mouse strain is not critical for cytokine and isotype induction by these adjuvants. Moreover, the fact that adjuvants seem to be capable of overcoming such an inherent IL-2/IFN-γ and IL-4 strain dichotomy and resulting in certain cytokine and antibody production makes them a powerful tool in manipulation of the immune response.

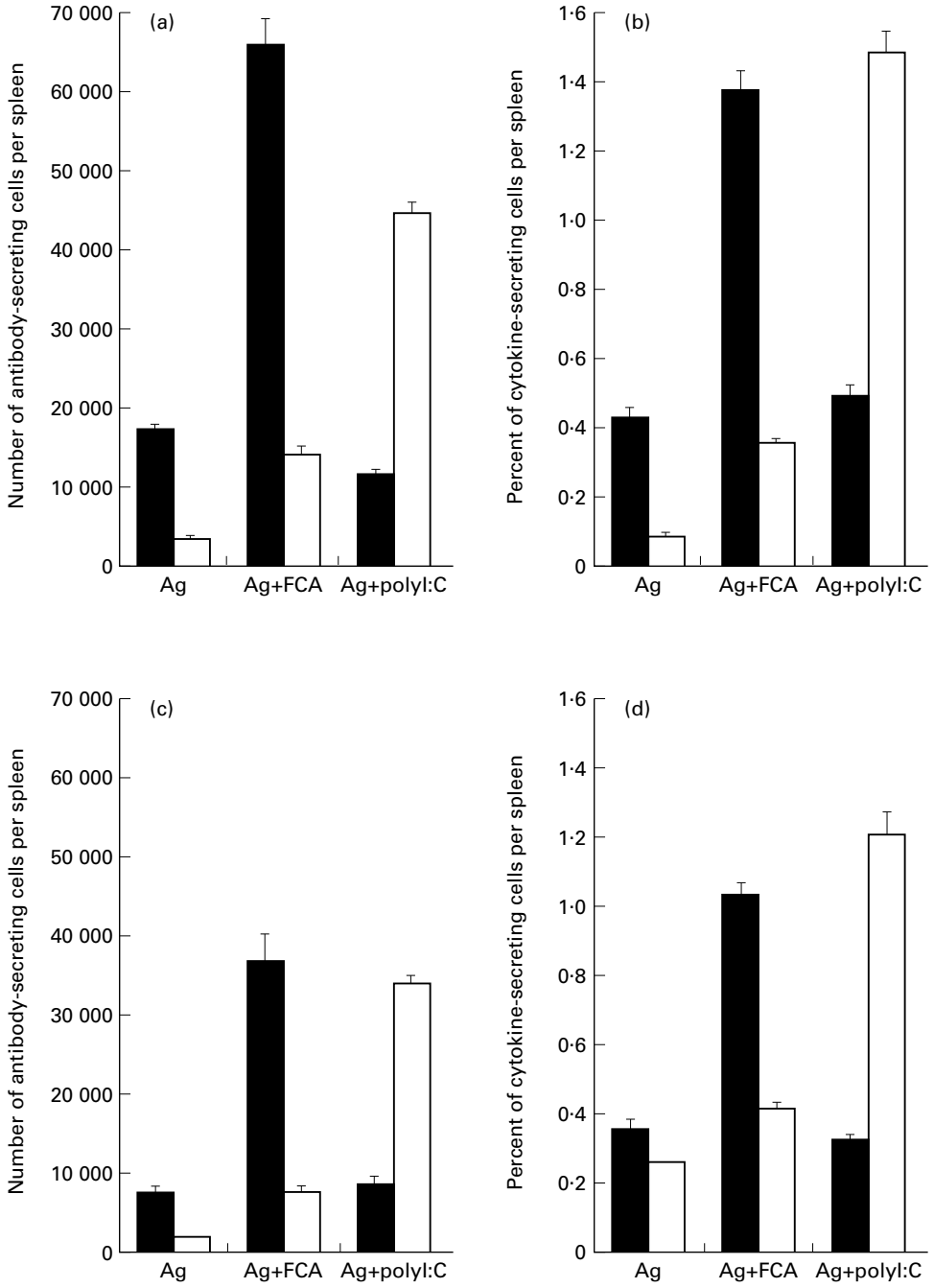

Fig. 5. The effect of Freund's complete adjuvant (FCA) and poly I:C on IgG1/IL-4 and IgG2a/IFN-γ production in BALB/c and C57Bl/6 mice. (a) IgG1 (■) and IgG2a (□) production in BALB/c, (b) IL-4 (■) and IFN-γ (□) production in BALB/c, (c) IgG1 (■) and IgG2a (□) production in C57Bl/6 and (d) IL-4 (■) and IFN-γ (□) production in C57Bl/6 mice. Results are expressed as mean ± s.e.m. of three independent experiments with three animals in each group.

It should be noted that the antigen (HGG–FITC) injected in the absence of adjuvant induces a type-2 response, increasing the production of IL-4, IL-5 and IL-13. This pattern changes when this antigen is injected with adjuvants that induce a different lymphokine profile, i.e. poly I:C. The observation that lymphokine production is dependent upon the adjuvants and not the antigen is consistent with our previous results [7,9], in which a decisive effect of non-specific stimuli-adjuvants on isotype switching has been reported. The present work is based on the use of a certain TD hapten–carrier complex that exerts a type-2 lymphokine gene expression and IgG1 antibody production. Our investigation will be extended to other haptin–carrier complexes as well as to antigens with type-1 lymphokine pattern and cytokine profile during secondary immunizations.

The present study demonstrates fundamental differences in the cytokine induction pattern according to the adjuvant used.

FCA, FIA, $Al(OH)_3$ and QuilA establish Th2 responses, enhancing IL-4, IL-13 and IL-5 production, while poly I:C exhibits Th1 responses, increasing IFN-γ, IL-2 and IL-6 production. Finally, poly A:U and $BeSO_4$ promote IL-5 and IL-6 production, while LPS and LiCl augment IL-6 and TNF-α production. Some of the cytokines induced are known to be directly involved in the isotype selection process as switching factors, while other cytokines act as post-switching differentiation factors. With the administration of the appropriate adjuvant, induction of a certain cytokine profile is achieved, resulting in antibodies of a specific isotype. In that way, the most efficient isotype of protective antibodies can be selected during vaccinations.

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