INTERLEUKIN-INDUCED INCREASE IN Ia EXPRESSION BY NORMAL MOUSE B CELLS

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The role of interleukins in B cell activation has been investigated in several experimental systems (1–7). At least with regard to proliferation and the generation of antibody-secreting cells, the nonspecific factors were without apparent effects on resting B cells, while they amplified responses of B cells previously "activated" by exposure to mitogens, anti-immunoglobulin (Ig)[1] antibodies, or antigen-specific/Ia-restricted helper T cell signals. Based on these observations, models have been developed in which the targets of lymphokines are B cells that have previously encountered specific antigen, which presumably induces expression or activates receptors for the relevant lymphokines.

These models of B cell activation parallel more definitive studies with T cells, where it has been demonstrated that mitogen or antigen/MHC activation of T cells leads to the expression of receptors for interleukin-2 (IL-2) rendering the cell sensitive to its growth-promoting effects (8, 9). Supporting the application of these observations to B cells is the finding that monoclonal antibodies directed against the IL-2 receptor complex detect its presence on the surface of B cells activated with lipopolysaccharide (LPS), but not on normal resting cells (9). The question that arises is whether these findings apply to the effects of interleukins on B cells in general or only to specific cases.

Numerous studies have demonstrated the importance of Ia in T cell collaboration with B cells in the generation of antibody responses (10–12). The requirement for I region identity may directly reflect requirements for delivery of an antigen-specific/I region–restricted helper T cell signal and indirectly the ability of B cells as well as macrophage (Mϕ) to present antigen to T cells in an MHC-restricted manner, leading to their production of B cell helper factors (3, 13–17). In this regard some studies have indicated that the level of B cell Ia

[1] *Abbreviations used in this paper:* AO, acridine orange; BCGF, B cell growth factor; Con A, concanavalin A; cOVA, chicken ovalbumin; FS6 Con A SN, Con A–induced culture SN of FS6-14.13; HBSS, Hanks' balanced salt solution; IFN$_\gamma$, interferon gamma; Ig, immunoglobulin; IL-2, interleukin 2; LPS, lipopolysaccharide; Mϕ, macrophage; MHC, major histocompatibility complex; P388 SN, P388D$_1$ culture SN; and SN, supernatant.

expression may be important to the efficiency with which T-B interactions occur (18, 19).

The level of Ia expression on normal B cells is very heterogeneous and may in part reflect the maturational state of the cells (20, 21). While the mechanisms controlling the level of Ia expression remain unknown, it has been established that B cell receptor cross-linking with anti-Ig antibodies will induce increased Ia expression both in vitro and in vivo (22, 23). Macrophage-derived monokines have also been implicated in the induction of Ia expression on density-fractionated bone marrow cells (24).

The evidence presented in this paper demonstrates that the level of Ia expression on normal resting B cells can be profoundly increased following exposure to T cell– and Mφ-derived interleukins, with kinetics similar to those seen with membrane Ig cross-linking agents. These observations suggest that receptors for these interleukins are expressed on resting B cells and their effects can be observed in the absence of secondary signals delivered by ligand-receptor Ig interactions and cognate T cell–B cell interactions.

Materials and Methods

Mice.   B6D2F$_1$ mice were bred in our facility from breeding stock obtained from The Jackson Laboratory, Bar Harbor, ME.

Antigens and Other Reagents.   Alpha-methyl-D-mannoside, chicken ovalbumin (cOVA), glutaraldehyde, hydroxyurea, lysine, and sodium azide were purchased from Sigma Chemical Co., St. Louis, MO; and concanavalin A (Con A) was purchased from Miles Laboratories, Inc., Elkhart, IN.

Trypsin (TPCK-treated, Worthington Biochemical Co., Freehold, NJ) digested cOVA was prepared as previously described (25).

B Cell Preparation.   2–4 d before sacrifice, mice were given an intraperitoneal injection of 0.4 ml of a 1:10 dilution of rabbit anti-mouse thymocyte serum (Microbiological Associates, Walkersville, MD). Spleen cell suspensions prepared from these mice in Hank's balanced salt solution (HBSS) were passed over Sephadex G10 columns (26), treated with a cocktail of antibodies for 30 min on ice, washed with HBSS, and then incubated with a 1:15 dilution of rabbit serum as the complement source (Grand Island Biological Co., Grand Island, NY) for 30 min at 37°C. The antibody cocktail was similar to that previously described (27) containing: B cell–absorbed rabbit anti-mouse thymocyte serum, T24/40.7 anti-Thy-1 monoclonal antibody (Dr. Ian Trowbridge, Salk Institute, La Jolla, CA), MK 2.2 anti-"Qa-like" monoclonal antibody, B16/146 anti-Qat-4 ascitic fluid (Dr. Ulrich Hammerling, Sloan-Kettering Memorial Cancer Institute, New York, NY), ADH4(15) anti-Lyt-2.2 ascites fluid (Dr. Paul Gottlieb, University of Texas, Austin, TX), and GK 1.5 anti-L3T4 monoclonal antibody (Drs. Deno Dialynas and Frank Fitch, The University of Chicago, Chicago, IL). Following antibody plus complement treatment the erythrocytes were lysed by treatment with ammonium chloride and viable cells enriched by centrifugation over Ficoll-hypaque (28).

B Cell Culture for Ia Induction.   1 × 10^6 T cell– and Mφ-depleted splenic B cells were cultured in 0.5 ml complete medium in Linbro 76-033-05 24-well culture plates (Flow Laboratories, Inc., McLean, VA). Culture conditions were modified from those of Mishell and Dutton (29, 30). Cultures contained: 10 mg/ml α-methyl-D-mannoside to prevent effects of residual Con A in nonspecific factor preparations and 2 mM hydroxyurea to inhibit DNA synthesis and cell division. All cultures contain 5% fetal bovine serum, from a lot selected for its ability to support in vitro anti-sheep erythrocyte plaque-forming cell responses with low backgrounds in control cultures. Interleukin-induced increases in B cell Ia expression of comparable magnitude have also been observed at fetal bovine serum concentrations of 0.01% (data not shown).

Nonspecific Factor Preparations.   Macrophage-derived helper factors were obtained as

the constitutive culture supernatant (SN) of the Mφ tumor cell line P388D₁ (P388 SN) (31). P388D₁ were cultured at 2×10^6 cells/ml in complete medium containing 1% fetal bovine serum. After 6 d of culture the cell free SN was harvested and stored at −20°C. This factor preparation has previously been shown to enhance the primary in vitro anti-sheep erythrocyte plaque-forming cell response of Mφ- and T cell–depleted splenic B cells (32).

An IL-2 and B cell growth factor (BCGF) containing SN were prepared from the mycoplasma free T cell hybridoma FS6-14.13 as previously described (FS6 Con A SN) (27, 33). FS6-14.13 at 10^6 cells/ml in complete medium containing 0.5% fetal bovine serum, were stimulated with 3 μg/ml Con A for 24 h. Based on the method of Watson et al. (34), the cell-free SN was made 0.1 M with α-methyl-D-mannoside and then $(NH_4)_2SO_4$ added to 40% saturation. The resulting precipitate was discarded and $(NH_4)_2SO_4$ added to 80% saturation. The second precipitate was resuspended in 0.15 M NaCl and then extensively dialyzed against 0.15 M NaCl and then against HBSS. The final concentration of the factor preparation was ~50× the original SN.

Gel filtration of FS6 Con A SN was performed using a 2.5×100 cm Sephadex G75 (Pharmacia Fine Chemicals, Uppsala, Sweden) column equilibrated with 0.1 M ammonium bicarbonate. 5 ml of FS6 Con A SN was applied to the column and eluted with 0.1 M ammonium bicarbonate. 10-ml fractions were collected, frozen, and lyophilized to remove water and ammonium bicarbonate. The elution profile (molecular weight vs. elution volume) of human gamma globulin, bovine serum albumin, chicken ovalbumin, chymotrypsin, myoglobin, and cytochrome c was used as a calibration standard.

Immunofluorescence Staining. After 24 h of culture the cells were washed in phosphate-buffered saline containing 1% fetal bovine serum and 0.2% sodium azide. The cells were then incubated with an appropriate dilution of a biotin-conjugated monoclonal anti-I-A[b,d] antibody D3.137.5.7 (Dr. Sue Tonkonogy, North Carolina State University School of Veterinary Medicine, Raleigh, NC) at 4°C for 20 min, at a cell concentration of 10^7/ml. The cells were then washed twice and incubated at 4°C for 20 min with a 1:100 dilution of fluoroscein-conjugated avidin (Vector Laboratories, Burlingame, CA). The cells were then washed twice and resuspended at 10^6/ml for analysis. The D3.137.5.7 monoclonal antibody was affinity purified by adsorption and elution from a staphylococcal protein A-Sepharose column. The resulting antibody was biotin conjugated using N-hydroxysuccinimidobiotin (Sigma Chemical Co., St. Louis, MO) (35).

Cytofluorometric Analysis. The relative fluorescence intensities of individual cells were measured using the Cytofluorograf System 50H (Ortho Diagnostic Instruments, Westwood, MA) equipped with a 5W argon laser. Forward narrow angle light scatter was used as a second parameter to facilitate exclusion of dead or aggregated cells from analysis. Fluorescence intensity data is presented as the log of integrated fluorescence.

Acridine Orange (AO) Cell Cycle Analysis. The staining procedure of Darzynkiewicz et al. (36, 37) was used throughout this study. This procedure involves incubation of cells, after mild EDTA-detergent treatment at low pH (0.1% Titron X-100, 1 mM EDTA, 0.1 N HCl, pH 3.8) with an aqueous solution of AO (13 μM). Due to the differential binding characteristics of AO to single- and double-stranded nucleic acids, DNA fluoresces green while RNA fluoresces red upon excitation by 488 nm laser light. Relative integrated red and green fluorescence were determined using the Cytofluorograf System 50H in standard configuration.

Cells that were dead before analysis were excluded from the assay based upon integrated forward light scatter and integrated green (DNA) fluorescence. Aggregated cells were discriminated and excluded based upon the ratio of peak green fluorescence and area of green fluorescence. To determine the proportion of cells that had entered cycle, histograms depicting relative red fluorescence (RNA) were constructed and integrated.

Preparation of Anti-Fab. Rabbit anti-mouse Ig was raised by subcutaneous injection of 100 μg of Fab fragments of normal mouse Ig in complete Freund's adjuvant. Anti-Fab antibodies were affinity purified using normal mouse IgG-Sepharose and eluted using 3.5 M $MgCl_2$. The resulting antibody was passed over a staphylococcal protein A-Sepharose column. The bound protein was eluted with 0.1 M sodium acetate, pH 4.3. Pepsin

digestion of the resulting anti-Fab antibody was accomplished using standard methods (38). Purified F(ab')$_2$ fragments were isolated by passing the neutralized digest over the staphylococcal protein A-Sepharose column, which retained the undigested Ig.

BCGF Assay.   The assay for BCGF was similar to that previously described (6). B cells were prepared as described above and cultured at 7.5 × 10^4/well in 96-well microtiter culture plates (Flow Laboratories, McLean, VA) using complete Mishell-Dutton medium. Triplicate cultures containing serial dilutions of the putative BCGF containing SN were stimulated for 72 h with 10 μg/ml rabbit F(ab')$_2$ anti-mouse Ig (not affinity purified) in the presence of 20% P388 SN and 10 mg/ml α-methyl-D-mannoside. Cultures were then pulsed with [^{3}H]thymidine for 4 h and incorporation determined by liquid scintillation counting.

Assay of IL-2 Activity.   The T cell growth–stimulating activity of IL-2 was assayed as previously described using the IL-2–dependent T cell line HT-2 (16, 25, 27, 33). Triplicate cultures containing 100 μl twofold dilutions of a putative IL-2–containing SN received 4,000 viable HT-2 cells. After 24 h the cultures were pulsed with [^{3}H]thymidine for 4 h and incorporation was determined by liquid scintillation counting.

Assay of Antigen Presentation by B Cells.   The assay for antigen-specific, Ia-restricted B cell stimulation of IL-2 production by the T cell hybridoma DO-11.10 (cOVA/I-A^d) was performed as previously described (25, 39). 1 × 10^5 DO-11.10 hybridoma T cells were cultured in 96-well microtiter tissue culture plates containing 1 mg/ml native cOVA or trypsin-digested, denatured cOVA and the indicated number of B cells in 0.3 ml complete medium. Cultures were incubated for 24 h at 37°C, whereupon the presence of IL-2 in the culture SN was determined using the IL-2–dependent T cell line HT-2. IL-2 titers were determined by titration of the SN and the first twofold dilution to yield <90% viable HT-2 cells upon visual inspection was defined as containing 1 U of IL-2.

For fixation, the B cells were washed twice with HBSS and adjusted to a final concentration of 5 × 10^6 cells/ml in HBSS. Glutaraldehyde was added to a final concentration of 0.05%. After 30 min at room temperature, fixation was stopped by addition of an equal volume of 0.2 M lysine in HBSS, pH 7.4. The cells were centrifuged and washed before use. Antigen-presenting cells fixed in this manner are unable to present native cOVA to T cells, but will present trypsin-digested, denatured cOVA (25).

Results

Effects of Interleukins on B Cell Ia Expression.   Previous studies have demonstrated that the constitutive culture SN of the Mϕ tumor cell line P388D$_1$ and the Con A–induced culture SN of the T cell hybridoma FS6-14.16 contain nonspecific helper factors that amplify B cell proliferative responses induced with anti-Ig antibodies as well as the generation of antigen-specific and polyclonal antibody-secreting cell responses upon appropriate stimulation (27, 32, 33, 40–42). In each of these systems the nonspecific factor preparations were without apparent effects on B cells when tested alone. We have now demonstrated that interleukins present in P388 SN and FS6 Con A SN will directly stimulate an increase in Ia expression on normal resting B cells.

B6D2F$_1$ splenic B cells that were depleted of Mϕ (and B cell blasts) by passage through Sephadex G10 columns and rigorously depleted of T cells by treatment with anti-thymocyte serum in vivo and a cocktail of antibodies in vitro, were cultured for 24 h alone or in the presence of P388 SN and/or FS6 Con A SN. After culture relative Ia expression was determined by staining with a biotin-conjugated anti-I-A^{b,d} monoclonal antibody followed by fluorescein-conjugated avidin and cytofluorometric analysis. The results of a representative experiment are shown in Fig. 1. The level of Ia expression in the control populations of B cells was extremely heterogenous, covering nearly three decades. In the presence
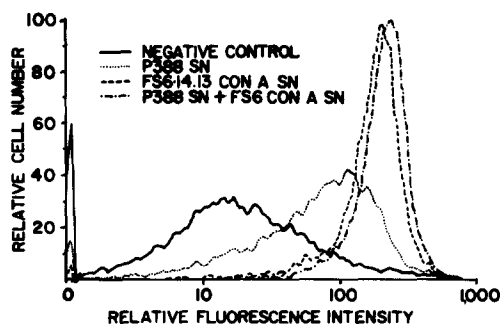
FIGURE 1. Interleukin-induced increase in B cell Ia expression. Normal splenic B cells were cultured for 24 h either alone or in the presence of P388 SN and/or FS6 Con A SN. Cells were stained with biotin-conjugated anti-I-A^{d,b} monoclonal antibody followed by fluorescein-conjugated avidin and analyzed by flow cytometry. 5,000 cells were analyzed for each histogram.
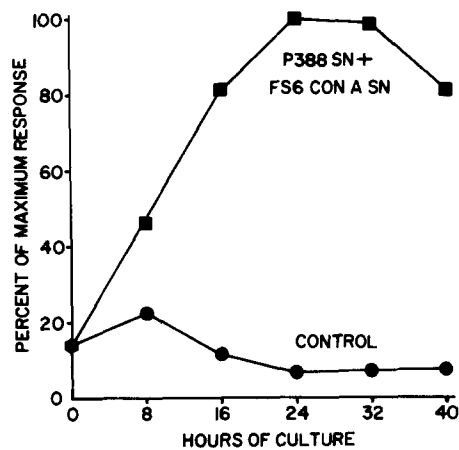

FIGURE 2. Kinetics of interleukin-induced increase in B cell Ia expression. Normal splenic B cells were cultured alone (control) or with P388 SN plus FS6 Con A SN. At the indicated time points the cells were stained with biotin-conjugated anti-I-A^{b,d} monoclonal antibody followed by fluorescein-conjugated avidin and then analyzed by flow cytometry. Relative mean fluorescence (Ia) was determined for each population and expressed as a percentage of the maximum response.

of P388 SN and/or FS6 Con A SN there was a reproducible increase in mean Ia expression of the B cell population relative to the control. In six consecutive experiments the increase in mean Ia expression induced with P388 SN was 4.9 ± 0.9, with FS6 Con A SN 10.7 ± 1.5, and with both preparations 13.0 ± 1.7. The effects of the two nonspecific preparations in combination were additive at best and not synergistic. Of particular note is the fact that there is only a small overlap in the fluorescence intensity of control B cell and cells cultured with FS6 Con A SN. This result suggests that virtually all B cells, not just a subpopulation of the cells were stimulated to increased Ia expression.

Kinetic Analysis of Increase in Ia Expression. Fig. 2 depicts a kinetic analysis of the relative change in mean Ia expression by the B cells. At the indicated time points the B cells were removed from culture, stained for Ia expression, fixed

with formaldehyde (3.36% final), and stored in the dark until cytofluorometic analysis. Control studies demonstrated that this fixation procedure has a minimal effect on autofluorescence and immunofluorescent staining. As shown in Fig. 2 the combination of P388 SN and FS6 Con A SN induced a rapid increase in mean Ia expression that was optimal at 24 h of culture. The mean Ia expression of the control population remained relatively stable, with only a small drop in Ia expression during the culture period. Analysis of Ia expression was routinely conducted at 24 h of culture. The stability of Ia expression in control cultures and the high viability of recovered cells (generally >90%) suggests that the effects of the interleukin preparations represent a true increase in B cell Ia expression and cannot be explained by selective survival or enrichment of B cells with high Ia expression.

Titration of P388 SN and FS6 Con A SN. In an attempt to demonstrate dose-response relationships for the interleukin preparations, a series of titration experiments were conducted. A representative experiment depicting the relative increase in Ia expression as a function of the SN volume added per culture is shown in Fig. 3. The results established that the log of mean Ia expression was linearly related to the log of the FS6 Con A SN or the P388 SN volume added per culture.

Relationship between Cell Cycle State and Increased Ia Expression. We next examined the effects of F(ab')$_2$ anti-Ig antibodies and the nonspecific factor preparations on increased Ia expression and entry into cell cycle. Entry into G$_1$ was assessed by changes in RNA content (red fluorescence) following staining with AO. To quantitate the proportion of cells that had entered cycle, histograms relating cell frequency to red fluorescence (RNA) were constructed (Fig. 5). Those cells containing elevated red fluorescence intensity to the right of the channel marker were considered to have entered G$_1$. These results are presented in Table II. Table I lists the mean Ia expression of the B cells relative to the control which did not receive anti-Ig or factors. Histograms depicting the profile of Ia expression from the four corner groups of Table I are shown in Fig. 4 and histograms depicting RNA content from these same groups in Table II are shown in Fig. 5.
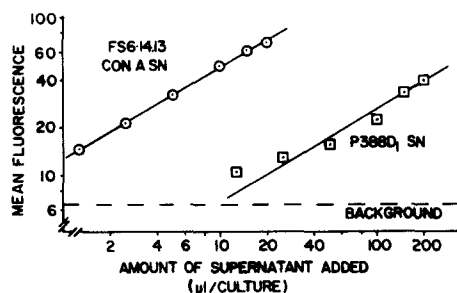

FIGURE 3.   Dose-response relationship of interleukin-induced increase in B cell Ia expression. Normal splenic B cells were cultured in 0.5 ml medium containing the indicated volume of P388 SN or FS6 Con A SN. At 24 h of culture cells were stained for surface Ia expression using biotin-conjugated anti-I-A^{b,d} monoclonal antibody, followed by fluorescein-conjugated avidin and analyzed by flow cytometry. Relative mean fluorescence (Ia) of each population is presented.

TABLE I

Relationship between Cell Cycle State and Increased Ia Expression:
Relative Mean Fluorescence (Ia) of B Cells Cultured with the
Indicated Interleukin Preparations and/or Anti-Ig

Culture SN added	F(ab')$_2$ anti-mouse Ig (μg/ml)			
	None	0.2	1.0	5.0
None	1.0	1.0	1.9	3.9
P388 SN (A)	4.3	4.7	7.1	10.2
FS6 Con A SN (B)	11.1	12.3	15.4	18.2
A + B	13.9	15.5	16.5	18.9

TABLE II

Relationship between Cell Cycle State and Increased Ia Expression:
Percentage of Cells Entering G$_1$ as Determined by Red Fluorescence
following Staining with AO*

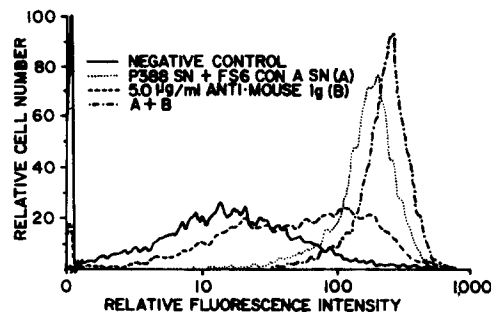| Culture SN added	F(ab')$_2$ anti-mouse Ig (μg/ml)			
	None	0.2	1.0	5.0
None	5.1	10.5	19.5	43.9
P388 SN (A)	17.5	22.2	45.3	53.8
FS6 Con A SN (B)	12.7	21.7	49.2	64.0
A + B	27.4	38.4	68.8	79.3

* See Fig. 5.

FIGURE 4. F(ab')$_2$ anti-mouse Ig and interleukin-induced increase in B cell Ia expression. Normal splenic B cells were cultured either alone or with 5.0 μg/ml rabbit F(ab')$_2$ anti-mouse Ig and/or P388 SN plus FS6 Con A SN. At 24 h of culture cells were stained for membrane Ia expression and analyzed by flow cytometry. 5,000 cells were analyzed for each histogram. (See Table I for relative mean fluorescence of each population).

As previously demonstrated (22, 23, 43), F(ab')$_2$ anti-Ig antibodies stimulated both increased Ia expression and entry into G$_1$ as indicated by increased RNA content. The addition of P388 SN and/or FS6 Con A SN further increased the magnitude of anti-Ig effects in an additive manner. The factors alone appear to stimulate a slight increase in cellular RNA content.

Two features of these results are of particular interest. First, the symmetry of the histogram depicting RNA content of the control population (Fig. 5) indicates that virtually all the cells are in G$_0$, suggesting that the interleukin preparations
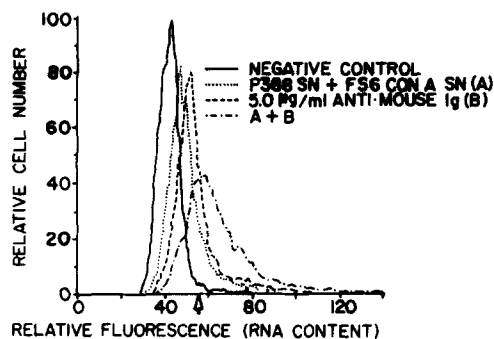
FIGURE 5. Effect of F(ab')₂ anti-mouse Ig and interleukins on entry into cell cycle. Histograms depict relative red fluorescence (RNA content) of normal splenic B cells stained with acridine orange following 24 h culture either alone or with 5.0 μg/ml rabbit F(ab')₂ anti-mouse Ig and/or P388 SN plus FS6 Con A SN. Each histogram was constructed by analysis of 5,000 cells by flow cytometry. Those cells exhibiting greater red fluorescence than the channel indicated by the arrow were considered to have entered cycle (see Table II).

were able to directly induce increased Ia expression on resting B cells in the apparent absence of other activating signals. This conclusion is further supported by the results when we deliberately tried to activate the B cells with anti-Ig antibodies. While the anti-Ig antibodies did cause an additional increase in the level of Ia expression on B cells cultured in the presence of P388 SN and FS6 Con A SN, the frequency of cells demonstrating increased Ia was not substantially higher. As compared to the nonspecific factor preparations, F(ab')₂ anti-Ig antibodies were better able to induce entry into cell cycle, but were less effective at inducing increased Ia expression. This dichotomy was even more apparent when the F(ab')₂ anti-Ig antibodies were used as a pulse before the initiation of culture (data not shown).

Analysis of Active Component(s) of FS6 Con A SN. Previous studies have established that Con A–induced culture SN of the T cell hybridoma FS6-14.13 contain at least two lymphokines, IL-2 and BCGF (44). In an attempt to determine whether either of these factors was involved in the induction of increased B cell Ia expression, FS6 Con A SN was fractionated on a Sephadex G75 column. Pooled fractions were then assayed for IL-2 activity (using the IL-2–dependent T cell line HT-2), for BCGF (using splenic B cells stimulated with low doses of rabbit F(ab')₂ anti-mouse Ig), and for their ability to induce increased B cell Ia expression. In each case the pooled fractions were titrated and units of activity/milliliter were determined: 1 U of activity was defined as the SN volume required to give a response equal to one-half the square root of the maximum response multiplied by the background response. The results of this analysis are shown in Fig. 6. The bulk of the IL-2 activity appeared in fraction I, corresponding to a molecular weight range of 35,000 to 58,000. The bulk of the BCGF and Ia-inducing activity appeared in fractions III and IV, corresponding to a molecular weight range of 9,300 to 26,000. The close correlation between Ia-inducing activity and BCGF activity and the lack of correlation with IL-2 activity suggests that BCGF or an as yet undefined T cell lymphokine may be responsible for inducing increased Ia expression on B cells.

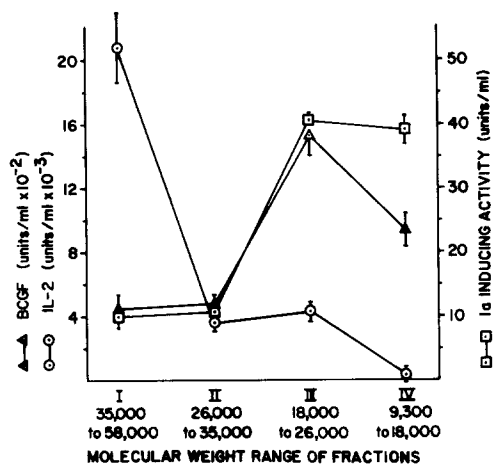*Role of IL-2 and Interferon-gamma (IFN_γ) in Enhanced B Cell Ia Expres-*

FIGURE 6. Sephadex G75 column fractionation of FS6 Con A SN, correlation of IL-2, BCGF, and Ia inducing activity. A Con A–induced cultured SN of the T cell hybridoma FS6-14.13 was fractionated on a Sephadex G75 column and fractions corresponding to the indicated molecular weights were then pooled. Each pooled fraction was then titrated for IL-2 (T cell growth factor activity using HT-2 cells), BCGF (proliferation of normal B cells stimulated with rabbit F(ab')₂ anti-mouse Ig) and Ia inducing activity, each expressed as units of activity/ml.

sion. Previous studies have established the ability of IFN$_\gamma$ to induce Ia expression by Mϕ (45, 46). In this regard we tested the ability of murine IFN$_\gamma$, prepared by recombinant DNA technology (kindly provided by Genentech, Inc., San Francisco, CA), to induce Ia expression in B cells. In addition we attempted to confirm the conclusion that IL-2 was not responsible for the B cell Ia enhancing activity of FS6 Con A SN by testing the effects of cloned human IL-2, also prepared by recombinant DNA technology (kindly provided by David Mark of Cetus, Emeryville, CA).

The results of these studies are shown in Fig. 7. FS6 Con A SN was used as the positive control and the data were plotted as a function of the units/milliliter BCGF activity in this preparation. Neither the cloned IFN$_\gamma$ nor the cloned IL-2 had detectable B cell Ia-inducing activity at concentrations as high as 1,000 U/ml (units IFN$_\gamma$ activity based on inhibition of viral replication and units of IL-2 based on T cell growth factor activity with HT-2 cells). The units of cloned IL-2 used in this experiment exceed the range of IL-2 activity present in FS6 Con A SN. In addition both the cloned IL-2 and cloned IFN$_\gamma$ failed to have effects on B cell Ia expression at concentrations where they can be shown to amplify the generation of anti-hapten and anti-sheep erythrocyte antibody responses (plaque-forming cells) by B cells prepared in an analogous manner (reference 44 and unpublished). These results suggest that the induction of increased B cell Ia expression is not mediated by IL-2 or IFN$_\gamma$.

Induction of Increased B Cell Ia Expression and Its Effect on Antigen-presenting Capacity. In an attempt to determine whether the interleukin-induced increase in B cell Ia expression might influence T-B interactions, we tested the ability of induced and control B cells to present antigen to antigen-specific/Ia-restricted T cell hybridomas. Fig. 8 depicts a representative experiment. B cells were cultured for 24 h either alone or in the presence of P388 SN and FS6 Con A SN and
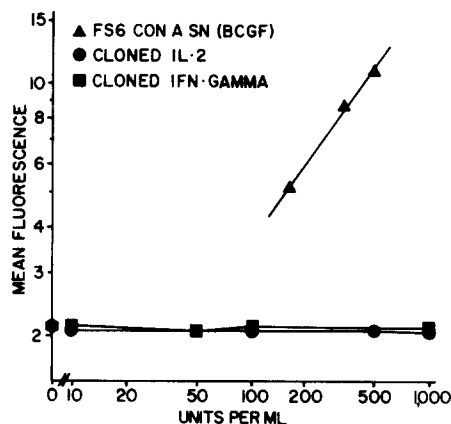
FIGURE 7.   Effects of cloned human IL-2 and cloned murine IFN$_\gamma$ on B cell Ia expression. Normal splenic B cells were cultured for 24 h either alone, with FS6 Con A SN, cloned human IL-2, or cloned murine IFN$_\gamma$. Cells were then stained for surface Ia expression and analyzed by flow cytometry. Data are expressed as the relative mean fluorescence (Ia) for each population as a function of U/ml of the lymphokine present in culture. U/ml of cloned IL-2 determined by T cell growth factor activity with HT-2 cells; IFN$_\gamma$ activity based on inhibition of viral replication; and FS6 Con A SN activity based on BCGF activity using anti-Ig stimulated B cells.

then titrated for their ability to present cOVA to the T cell hybridoma DO-11.10 (OVA/I-A^d). Presentation of antigen was monitored by the production of IL-2 by the T cell hybridoma. The lower panel of Fig. 8 demonstrates that B cells incubated with P388 SN plus FS6 Con A SN (14-fold increase in Ia expression) are more efficient in presenting cOVA to the T cell hybridoma DO-11.10 than the control B cells. Similar results were obtained with three other T cell hybridomas, differing in antigen/MHC specificity.

Clearly, there could be a variety of changes induced by the interleukins that might contribute to this increase in antigen-presenting ability. In an attempt to eliminate the potential contribution of changes in intracellular antigen-processing events, we took advantage of the recent demonstration that glutaraldehyde-fixed accessory cells are capable of MHC-restricted presentation of antigens that have been "processed" in vitro by chemical or enzymatic degradation (25). As shown in the upper panel of Fig. 8 using DO-11.10 and trypsin-digested, denatured OVA, the enhanced antigen-presenting capacity of B cells cultured with P388 SN plus FS6 Con A SN relative to control cells was also apparent following glutaraldehyde fixation. While this result does not conclusively demonstrate that the increase in Ia expression is causally related to the increase in efficiency of antigen presentation, it is consistent with this possibility.

Discussion

The data presented in this paper demonstrate that the level of Ia expression on normal resting B cells can be dramatically increased by exposure to interleukins present in the constitutive culture SN of the Mϕ tumor line P388D1 and the Con A–induced culture SN of the T cell hybridoma FS6-14.13. When tested individually, both these nonspecific factor preparations increased B cell Ia
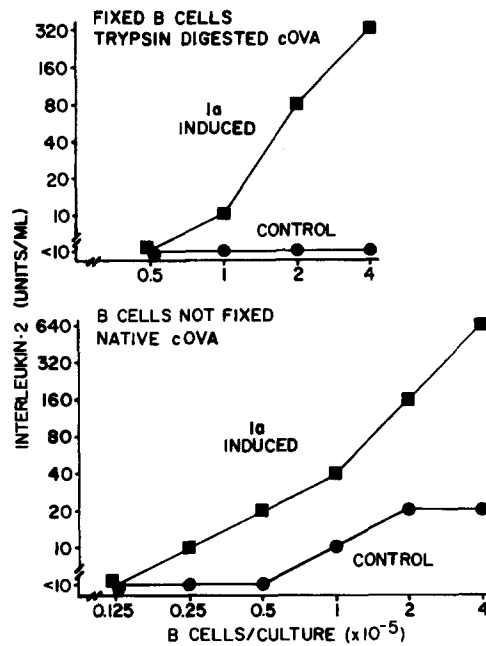
FIGURE 8. Enhanced antigen-presenting capacity of normal B cells incubated with interleukin preparations. Normal splenic B cells were cultured for 24 h either alone (control) or with P388 SN plus FS6 Con A SN (Ia induced). Cultures did not contain hydroxyurea. Relative to the control there was a 14× increase in mean fluorescence (Ia) of the B cells incubated with the nonspecific factor preparations. The two populations of B cells were then titrated for their ability to present cOVA to the T cell hybridoma DO-11.10 (cOVA/I-A^d), leading to the production if IL-2. *Lower panel:* units of IL-2 produced by DO-11.10 following stimulation with native cOVA and the indicated number B cells (not fixed). *Upper panel:* Units of IL-2 produced by DO-11.10 following stimulation with trypsin digested, denatured cOVA and the indicated number of glutaraldehyde-fixed B cells.

expression in a dose-dependent manner and when tested in combination at subsaturating concentrations, their effects were not synergistic, but additive. This interleukin (P388 SN + FS6 Con A SN)-induced increase in surface Ia expression was observed to occur in virtually all the B cells, resulting in a 13-fold increase (average of 6 consecutive experiments) in relative mean fluorescence of the population following staining for surface Ia.

The ability of the nonspecific factor preparations to induce increases in resting B cell Ia expression appeared to be a direct effect of the interleukins in the absence of additional activating signals. This conclusion is supported by two observations. First, although cell cycle analysis demonstrated that 95% of the B cells not exposed to the interleukins remained in G$_0$ throughout the culture period, virtually all exhibited increased Ia expression following exposure to the interleukin preparations. Second, the rate of interleukin-induced increase in Ia expression was extremely rapid, apparently occurring as quickly as new transcription, translation, and expression could occur (47). If an additional signaling event were prerequisite for the interleukin effect, one might have expected to see a longer lag before the increase in Ia was detectable.

These observations have important implications for models regarding the

effects of interleukins on B cell activation. First, they imply that functional receptors for at least some interleukins exist on the surface of normal resting B cells. Second, that these interleukins have direct effects on B cells (level of Ia expression and antigen-presenting capacity) in the absence of ancillary activating signals involving ligand-Ig receptor cross-linking. It is noteworthy in this regard that several investigators have described nonspecific T cell factors that appear to directly activate normal resting B cells (48, 49). The relationship of these observations to the effects of FS6 Con A SN on B cell Ia expression awaits further study.

The identity of the factors in P388 SN and FS6 Con A SN, which are responsible for enhancement of B cell Ia expression, have yet to be conclusively determined. The FS6 Con A SN is known to contain at least two lymphokines, IL-2 and BCGF (44). Column chromatography of FS6 Con A SN suggested a correlation exists between BCGF and the Ia-inducing activity on the basis of molecular weight. These two lymphokine activities have also been observed to be both qualitatively and quantitatively correlated in Con A SN from other T cell sources (data not shown). However, in the absence of a rigorous biochemical characterization it is not possible to definitively establish the nature of the T cell lymphokine involved in enhancing B cell Ia expression.

The Ia-inducing activity of FS6 Con A SN showed no correlation with IL-2, a result that was consistent with the fact that cloned human IL-2 had no effect on B cell Ia expression, at concentrations where it could be shown to enhance B cell antibody responses (unpublished). Similarly, cloned mouse IFN$_\gamma$ had no effect on the level of B cell Ia expression, when used at concentrations at which it could be shown to amplify B cell antibody responses (46). This latter observation was particularly interesting in view of the ability of IFN$_\gamma$ to induce enhanced Ia expression and antigen-presenting capacity in Mϕ (45, 46). Our observations suggest that a parallel system exists in B cells, which does not, however, involve IFN$_\gamma$. It is unclear at this time whether the interleukin-induced enhancement of B cell antigen-presenting capacity directly reflects the increase in Ia expression or whether other surface changes are also involved.

Historically, supernatants of P388D$_1$ have been used as a reference source of interleukin 1 activity (50). Attempts to demonstrate that a low molecular weight (~15,000 daltons) component of P388 SN was responsible for the B cell Ia-enhancing activity have been equivocal. Sephadex G75 chromatography of ammonium sulfate–precipitated P388 SN has demonstrated as small peak of activity with a molecular weight of ~15,000, however the bulk of activity appears in the void volume, corresponding to a molecular weight of >80,000. It is noteworthy in this regard that a high molecular weight factor produced by P388D$_1$ has been recently described, which induces Ia expression by the Mϕ tumor line WEHI 3 (51). Characterization of the factor active in our assay systems awaits further study.

Summary

The constitutive culture supernatant (SN) of the macrophage tumor line P388D$_1$ (P388 SN) and the concanavalin A (Con A)–induced culture supernatant of the T cell hybridoma FS6-14.13 (FS6 Con A SN) were shown to contain

nonspecific factors capable of inducing increased Ia expression by normal resting B cells in a dose-dependent manner. In six consecutive experiments the relative increase in Ia expression induced by P388 SN was 4.9 ± 0.9, with FS6 Con A SN 10.7 ± 1.5, and with a combination of both preparations 13.0 ± 1.7. This increase in Ia expression was observed to occur in virtually all the B cells, reaching maximum levels within 24 h of culture.

The interleukin-induced increase in B cell Ia expression occurred in the absence of ancillary signals provided by ligand-receptor Ig cross-linking and despite the fact that virtually all the control B cells, cultured in the absence of factors, remained in G_0. These results suggest that functional receptors for at least some interleukins are expressed on normal resting B cells and their effects can be manifest in the absence of additional activating signals. The increased Ia expression induced by the nonspecific factor preparations was shown to be correlated with enhanced antigen-presenting capacity by the B cells to T cell hybridomas.

The nature of the interleukins responsible for these effects remains to be definitively determined, however, the activity of FS6 Con A SN was shown to correlate with B cell growth factor activity and increased B cell Ia expression was not observed using interleukin 2 (IL-2) or interferon-gamma, prepared by recombinant DNA technology.

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