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Suppressive B-cell factor (SBF) produced by FcR_γ-bearing B cells; suppression of B, but not non-B-cell proliferation

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Summary. B cells that have receptors for the Fc portion of IgG (FcR_γ^+ B cell) elaborate an immunoregulatory lymphokine termed suppressive B-cell factor (SBF) after binding immune complexes, such as sheep erythrocytes sensitized with IgG anti-sheep erythrocyte antibody (EA). For producing SBF, *de novo* protein is required, but not DNA or DNA-dependent RNA synthesis. This mediator is released into the culture supernatant of FcR_γ^+ B cells during 6 to 48 hr after stimulation by EA. SBF suppresses the proliferation of B, but not non-B cells. Thus, it suppressed (i) plaque-forming cell responses in the induction phase in an antigen-non-specific manner, (ii) DNA synthesis of lipopolysaccharide-activated B cells, but neither concanavalin A nor phytohaemagglutinin-activated T cells, and (iii) the proliferation of B but not non-B tumour-cell lines by acting at the G_1-S junction in the cell cycle. Concordance of H-2 haplotype between SBF-producing mice and target B cells is necessary for the suppression.

Thus, the action of SBF is B-cell specific and antigen-non-specific. Immune complex-mediated negative feedback regulation seems to be operated by lymphokines such as SBF which may be also involved in the surveillance for B-cell tumours.

Abbreviations: FcR_γ, Fc receptor specific for IgG; FcR_γ^+ B cell, FcR_γ-bearing B cell; FcR_γ^- B cell, non-FcR_γ-bearing B cell; SBF, suppressive B-cell factor; HRBC, horse red blood cell; SRBC, sheep red blood cell; DNP-Asc, dinitrophenyl-conjugated *ascaris suum* extract; DNP-DE, dinitrophenyl-conjugated dextran T-2000; EA, erythrocyte sensitized with 7S rabbit anti-SRBC antibody; PFC, plaque-forming cell; LPS, lipopolysaccharide from *E. Coli.*; Con A, concanavalin A; PHA, phytohaemagglutinin; PBS, phosphate-buffered saline; MEM, minimum essential medium; FCS, foetal calf serum; MMC, mitomycin C; AcM-D, actinomycin D; PurM, puromycin.

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INTRODUCTION

A number of studies have demonstrated the presence of receptors for each class of Fc portion of immunoglobulins (Fc receptor, FcR) on various immunocompetent cell surfaces (Dickler, 1976) and some aspects of immune responses seem to be regulated by an interaction between FcR and the endproduct of immune responses, such as immune complexes (Uhr & Möller, 1968; Theofilopoulos & Dixon, 1979; Kölsch *et al.*, 1980).

In a series of studies on the immunological roles of FcR for IgG (FcR_γ) on murine lymphocytes, it has been demonstrated that splenic FcR_γ^+ B cells exert a suppressive effect on plaque-forming cell (PFC) responses in an antigen-non-specific manner, after sti-

mulation by immune complexes via FcR_γ (Masuda *et al.*, 1978). Important issues are that this type of suppression is mediated by the culture supernatant of FcR_γ^+ B cells, termed suppressive B-cell factor (SBF), but not of adherent macrophages or T cells (Masuda *et al.*, 1978), and that the target is B, but not helper T cells (Miyama, Yamada & Masuda, 1979). Our recent report which proved the *in-vivo* significance of SBF suggests that a lymphokine as SBF might be physiologically involved in the mechanism of feedback regulation of immune responses (Miyama-Inaba *et al.*, 1982). Physicochemical characteristics and the proof of physiological significance of immunoregulatory lymphokines, such as immunogloblin binding factor (IBF; Schimpl & Wecker, 1979) in addition to SBF, are indeed a matter of concern for the understanding of immune regulation.

In addition to showing that SBF is really synthesized *de novo* by FcR_γ^+ B cells, the current studies are intended to discover how SBF affects the proliferation of B cells in PFC and mitogen responses and tumour-cell lines of B-cell origin *in vitro*.

The results demonstrate that not only the response of normal B cells to LPS, as well as to antigen, but also the proliferation of tumour cells of B-cell origin are suppresssed by SBF; this implies the presence of an acceptor site for SBF on B cells.

MATERIALS AND METHODS

Mice

Inbred mice of BALB/c, C3H/He and DBA/2 strains of both sexes were used at an age of 12 weeks. Mice were maintained in the Institute of Experimental Animals, Faculty of Medicine, Kyoto University under specific pathogen-free conditions.

Antigen

Horse red blood cell (HRBC) and dinitrophenyl-conjugated *ascaris suum* extract (DNP-Asc) were used in *in-vivo* primary and secondary PFC responses, respectively. Dinitrophenyl-conjugated dextran (T-2000; DNP_{157}-DE) was used in *in-vitro* primary PFC response.

Antisera

The 7S fraction of rabbit anti-sheep red blood cell (SRBC) antiserum was separated from the hyper-immunized serum by gel chromatography on Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden). Anti-Thy-1.2 monoclonal antibody was purchased from Olac Ltd. (Bicester, Oxon., England).

Mitogens

Lipopolysaccharide from *E. Coli.* (LPS, 026:B6) was purchased from DIFCO Lab. (Detroit, M), phytohaemagglutinin (PHA) from E-Y Lab. (San Meteo, CA), and concanavalin A (Con A) from Pharmacia Fine Chemicals.

Media

Eagle's minimum essential medium (MEM) and RPMI 1640 was obtained from Nissui Seiyaku Co. Ltd., Tokyo, Japan.

Cell lines

WC-2 (AKR, thymoma, H-2^k), L-1210 (DBA/2, B lymphoma, H-2^d), P_3-NS1-Ag4-1, X63-Ag8.653, MOPC-31C, MOPC-315 (BALB/c, myeloma, H-2^d), X-5563 (C3H/He, myeloma, H-2^k), DL-4, DL-5, MLA (DBA/2 lymphoma, H-2^d), DL-1, DL-3, DL-8 (DBA/2, T lymphoma, H-2^d) and 3T3-A31 (BALB/c, fibroblast, H-2^d) were used. Cell-line cells of DL series and MLA were established from lymph nodes of DBA/2 mice bearing spontaneous lymphomas. DL-4, 5 and MLA expressed surface Ig, but not Thy-1 antigen, while DL-1,3 and 8 possessed Thy-1 antigen, but not surface Ig. The culture medium consisted of RPMI 1640 supplemented with 10% foetal calf serum (FCS; GIBCO, Grand Island, NY), penicillin (100 U/ml) and streptomycin (100 μg/ml). The cultures were maintained at 37° in an atmosphere of 5% CO_2.

Production of SBF

The spleen cell suspension (5×10^7/ml) was mixed with an equal volume of 10% SRBC coupled with a subagglutinating dose of IgG antibody (EA), agitated gently at 37° for 15 min, centrifuged at 100 ***g*** for 5 min, and followed by an incubation at 37° for further 45 min. FcR_γ^+ cells were separated from EA non-rosette-forming (FcR_γ^-) cells by a centrifugation on a cushion of Ficoll–Isopaque solution. The pellet (FcR_γ^+ cells) and the interface (FcR_γ^- cells) were re-rosetted and re-sedimented by the same procedure. The FcR_γ^+ cell fraction, more than 90% pure, was treated with anti-Thy-1.2 antibody and complement at 37° for 45 min to remove EA and T cells. The FcR_γ^- cell fraction, almost 100% pure, was also treated in the same way as this. Twenty-four hour-culture supernatants of 1×10^7/ml FcR_γ^+ and FcR_γ^- B cells in plain RPMI 1640 were termed SBF and FcR_γ^- B sup. respectively.

Modulation of the production of SBF

Pharmacologic modulation. The spleen-cell suspension (5×10^7/ml) was treated with following anti-metabolic agents at 37° for 30 min; 50 or 5 μg/ml mitomycin C (MMC; Kyowa Hakko Co. Ltd., Tokyo, Japan), 1 or 0·1 μg/ml actinomycin D (AcM-D; P-L Biochemicals Inc., Milwaukee, WI), and 10 or 1 μg/ml puromycin (PurM; MAKOR Chem. Ltd., Israel).

Irradiation. The spleen-cell suspension (1×10^7/ml) was irradiated with a soft X-ray machine (SOFTEX Co. Ltd., Tokyo, Japan) either at 130 R or 1000 R. The culture supernatant of FcR_γ^+ B cells obtained from spleen cells modulated thus was prepared by the method mentioned above. The viability of irradiated FcR_γ^+ B cells after the culture for 24 hr was almost the same as unmodified FcR_γ^+ B cells.

Mitogen response

Triplicate cultures of 5×10^5 spleen cells in 0·1 ml culture medium were started with either 0·1 ml SBF, FcR_γ^- B sup., or RPMI 1640 in 96-well flat-bottomed microculture plates (NUNC, Denmark). Each group of cultures was activated with one of the following mitogens: 50 μg/ml LPS, 5 μg/ml Con A, or 25 μg/ml PHA. These concentrations gave the maximal stimulation in dose–response preliminary tests. After 56 hr of incubation, the cultures were pulsed with 1 μCi [methyl-^{3}H]-thymidine ([^{3}H]TdR; 20 Ci/mM; Amersham, U.K.) and harvested 16 hr later to count [^{3}H]TdR incorporation by using a Packard liquid scintillation counter. The suppressive activity of SBF in mitogen responses was expressed as '% suppression' as follows: % suppression = (1 − [c.p.m. mitogen response with SBF − c.p.m. background]/[c.p.m. mitogen response without SBF − c.p.m. background]) × 100.

PFC response

In-vivo PFC response. The 5×10^7 spleen-cell pellet obtained from C3H/He mice primed with 10 μg alum precipitated DNP-Asc 6 weeks previously was treated with either 1 ml RPMI 1640 or culture supernatants of FcR_γ^+ B cells harvested at the various periods indicated, at 37° for 45 min. After washing twice, 1×10^7 cells were transferred into lethally irradiated (700 R whole body irradiation with a Toshiba RE 1024 X-ray machine) syngeneic recipients and challenged with 10 μg DNP-Asc. PFC assay were carried out on day 7 for secondary indirect anti-DNP PFC.

In order to test the effect of SBF on antibody-secreting cells, the 1×10^7 spleen-cell pellet obtained from C3H/He mice immunized with 4×10^8 HRBC 7 days previously was treated with either 1 ml SBF or RPMI 1640 at 37° for 45 min. Immediately after washing twice, PFC assay were performed for primary indirect anti-HRBC PFC.

In vitro PFC response. The 5×10^6 C3H/He spleen cells were cultured in the culture medium supplemented with 2ME (10^{-5} M) in 24-well culture plates (Limbro, New Haven, CI) in the presence (0·1 μg/well) or absence of DNP-DE. SBF derived from C3H/He mice was added to some cultures (50% vol:vol) at 0, 6, 12, 20 or 30 hr after the start of cultures. PFC assay was carried out on day 5 for primary direct anti-DNP PFC. The suppressive activity of SBF in PFC response was expressed as '% suppression' as follows: % suppression = (1 − [PFC with SBF − PFC background]/[PFC without SBF − PFC background]) × 100.

Effect of SBF on the proliferation of tumour-cell lines

Triplicate cultures of 1×10^3 tumour cells in 0·1 ml culture medium were started with either 0·1 ml SBF, FcR_γ^- B sup., or RPMI 1640 in 96-well flat-bottomed microculture plates (NUNC). The cell number of these cultures was counted by trypan blue-dye exclusion test every day for 4 days. The suppressive activity of SBF in the tumour-cell proliferation on day 4 was expressed as '% suppression' as follows: % suppression = (1 − [cell number with SBF]/[cell number without SBF] × 100.

Synchronizing culture of L-1210

According to the serum starvation method (Brooks, 1976), L-1210 cells in logarithmic growing phase were cultured in FCS-free RPMI 1640 supplemented with antibiotics. After 48 hr (0 hr), FCS was added to the culture to a final concentration of 10%. By the stimulation of FCS, L-1210 cells which had been in the G_0/G_1 phase under the serum-free condition resumed DNA synthesis and shifted to the S phase 12 hr after the addition of the serum, as judged by [^{3}H]TdR incorporation. Every 4 hr after the stimulation by FCS, 0·01 ml SBF was added to the culture of synchronized L-1210 cells in 96-well flat-bottomed microculture plates containing 1×10^4 cells/0·2 ml/well. Each culture was pulsed with 1 μCi of [^{3}H]TdR 4 hr before the harvest, and was harvested every 4 hr to determine the [^{3}H]TdR incorporation.

RESULTS

Kinetics of SBF production

Kinetics of SBF production by FcR_γ^+ B cells were analysed by using *in-vivo* adoptive secondary responses. Spleen cells transferred were pretreated at 37° for 45 min with culture supernatant of FcR_γ^+ B cells harvested at 6, 12, 24 and 48 hr after starting the culture. It took 2 hr from EA-rosetting to starting of the culture. FcR_γ^+ B cells cultured were washed at each time point and resuspended in fresh medium. Although the 0–6 hr culture supernatant of FcR_γ^+ B cells had no effect on indirect anti-DNP PFC response, the 6–12 and 12–14 hr culture supernatants suppressed PFC number by 48·0% and 46·5% respectively, as shown in Table 1. Significant suppression was attained by the 24–48 hr culture supernatant and the release of SBF continued for at least 48 hr.

These findings confirm our previous observation that SBF affects the activity of precursor B cells and suggest that it may be *de novo* synthesized after stimulation of FcR_γ with immune complexes.

Time course of the suppressive effect of SBF on *in vitro* antibody responses

In *in-vitro* antibody responses where SBF had been added to the cultured spleen cells (50% vol:vol), it suppressed the PFC response completely (Fig. 1). It was noteworthy that the suppressive effect was depen-

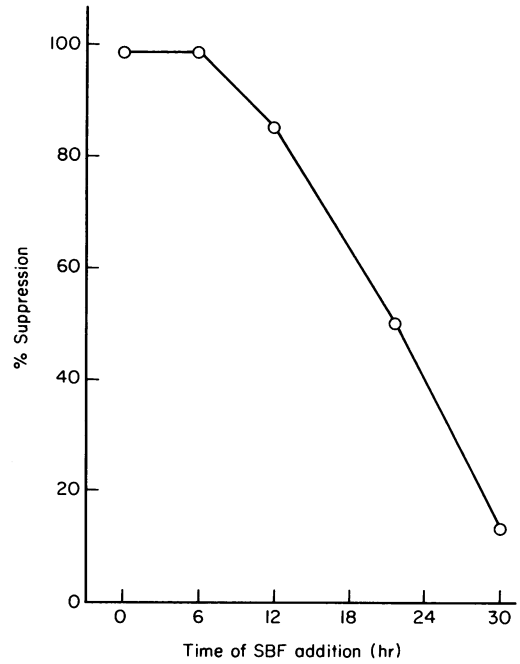

Figure 1. Time course of the suppressive effect of SBF on *in-vitro* primary anti-DNP responses. Spleen cells were cultured with or without 0·1 μg DNP-DE, and SBF was added at indicated time after the start of cultures. Data are expressed by mean % suppression of triplicate cultures, as compared with the control response, cultured in the absence of SBF (6200 ± 600 PFC/10^7 spleen cells).

Table 1. Kinetics of SBF production

Donor cells transferred	Treatment of cells*	Indirect anti-DNP PFC/spleen†	% Suppression‡
DNP-ASC primed spleen cells $1{\cdot}0 \times 10^7$	Media	$46{,}750 \pm 2808$	
	0–6 hr culture sup of FcR_γ^+ B cells	$54{,}062 \pm 4412$	0
	6–12 hr culture sup of FcR_γ^+ B cells	$24{,}300 \pm 1295$§	48·0
	12–24 hr culture sup of FcR_γ^+ B cells	$25{,}000 \pm 2038$§	46·5
	24–48 hr culture sup of FcR_γ^+ B cells	$33{,}450 \pm 2072$¶	28·4

* Pellets of 5×10^7 spleen cells of C3H/He mice immunized with 10 μg DNP-Asc were treated with 1 ml RPMI 1640 or culture supernatants of C3H/He. FcR_γ^+ B cells were harvested at intervals as shown in the Table. It took 2 hr from EA-rosetting to starting of the culture. FcR_γ^+ B cells were washed twice and resuspended in fresh medium at every time harvested.

† Mean PFC number ± standard error of five mice.

‡ Mean % suppression as compared with the control PFC response.

§ $P < 0{\cdot}001$, as compared with the control response.

¶ $P < 0{\cdot}01$.

dent on the time of SBF addition: the significant suppression was obtained only when SBF was added by 12 hr after the start of the culture. Supplementing with SBF 24 hr after the start of the culture resulted in no effect on the PFC responses at all.

In addition, in the system of *in-vivo* primary PFC responses to HRBC, the antibody response on day 7 was not affected by the treatment of antibody-producing effector cells with SBF immediately before the PFC assay (data not shown).

These results indicate that SBF exerts the suppressive effect in the induction phase of the antibody response, but not the effector phase.

Suppressive effect of SBF on mitogen responses

Since SBF exerted its suppressive activity in the induction phase of the antibody response, the effect of SBF on DNA synthesis was examined by mitogen responsiveness. SBF derived from BALB/c mice suppressed the LPS response of syngeneic spleen cells in a dose-dependent manner; 50% suppression by 1:100 diluted SBF. However, SBF did not suppress Con A and PHA responses, and FcR_γ^- B sup. had no effects on any mitogen response (Fig. 2).

These findings demonstrate that in mitogen responses SBF suppresses DNA synthesis of B but not of T cells, indicating that the suppression by SBF is B-cell specific.

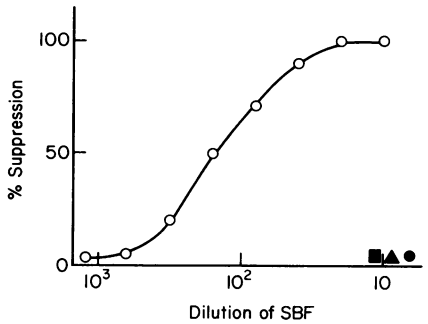

Figure 2. Suppressive effect of SBF on mitogen responses. BALB/c spleen cells were cultured with reciprocally diluted SBF derived from BALB/c mice with or without 50 μg/ml LPS (○), 5 μg/ml Con A (■), or 25 μg/ml PHA (▲). Data are shown with mean % suppression of triplicate cultures as compared with control response, in the absence of SBF. These control responses were 21,000±1400 (LPS), 38,200±3250 (Con A), and 28,660±2505 c.p.m. (PHA). Mitogen responses were not affected by FcR_γ^- B sup. (●).

Effect of SBF on the proliferation of tumour-cell lines

Since SBF suppressed both antibody and LPS responses by affecting B cells directly, experiments were expanded to examine the possible suppressive effect of SBF on *in-vitro* proliferation of B-cell tumour lines in comparison with non-B-cell lines (Table 2). The proliferation of B-cell tumour lines, such as L-1210, DL-4, DL-5, and MLA, was significantly suppressed when cultured with SBF derived from syngeneic mice. This suppression was also observed when tumour cells were pretreated with SBF (data omitted) as in the case of PFC responses (Table 1). On the other hand, SBF failed to suppress the proliferation of non-B-cell lines. FcR_γ^- B sup. which had no suppressive effect on both antibody (Miyama *et al.*, 1979) and LPS responses of spleen cells (Fig. 2), also had no effect on the proliferation of these cell-line cells.

Furthermore, it was noted that H-2 haplotype between mice providing SBF and tumour cells should be matched for the successful supression of the proliferation as observed in the case of PFC (Miyama *et al.*, 1979) and LPS responses (Suzuki *et al.*, submitted). That is, SBF derived from BALB/c mice ($H\text{-}2^d$) suppresses the proliferation of L-1210 cells ($H\text{-}2^d$), but not of X-5563 cells ($H\text{-}2^k$), while SBF derived from C3H/He ($H\text{-}2^k$) suppresses the proliferation of X-5563 cells, but not of L-1210 cells.

Thus, SBF is thought to be a suppressive lymphokine against the proliferation of activated B cell—antigen or mitogen-stimulated B cell and B-cell tumour lines.

Cell cycle dependency of the effect of SBF

Synchronized L-1210 cells were used to determine on which phase of the cell cycle SBF exerted a suppressive activity. As shown in Fig. 3, the DNA synthesis was blocked when SBF derived from BALB/c mice was added to cultures 4 to 12 hr after the supplement of the serum (0 hr). However, when SBF was added 16 hr or later it gave little suppressive effect on the DNA synthesis.

This implies that SBF exerts its suppressive effect on the proliferation of L-1210 cells at the G_1-S junction of the cell cycle.

Modulation of the production of SBF

In order to clarify the requirement of *de novo* DNA, RNA or protein synthesis for the production of SBF, FcR_γ^+ B cells were prepared from spleen cells which

Table 2. Effect of SBF on the proliferation of tumour-cell lines

Cells	Types	Strain	H-2(SBF)	% Suppression (day 4) SBF	% Suppression (day 4) FcR_γ^- B sup.
L-1210	B	DBA/2	*d*	95	0
DL-4	B	DBA/2	*d*	60	0
DL-5	B	DBA/2	*d*	80	0
MLA	B	DBA/2	*d*	85	0
X-5563	B*	C3H/He	*k*	90	0
MOPC-31C	B*	BALB/c	*d*	100	0
MOPC-315	B*	BALB/c	*d*	90	0
P_3-NS1-Ag4-1	B*	BALB/c	*d*	100	0
X63-Ag8.653	B*	BALB/c	*d*	95	0
L-1210	B	DBA/2	*k*	0	0
X-5563	B*	C3H/He	*d*	0	0
WC-2	T*	AKR	*k*	0	0
DL-1	T	DBA/2	*d*	0	0
DL-3	T	DBA/2	*d*	0	0
DL-8	T	DBA/2	*d*	20	0
3T3-A31	non-T,B	BALB/c	*d*	0	0

Tumour cells shown in the Table were cultured with SBF or FcR_γ^- B sup. SBF and FcR_γ^- sup. used was derived from BALB/c (H-2^d) or C3H/He (H-2^k) mice. Data are expressed as mean % suppression of the cell growth as compared with the control culture on day 4. B* are myeloma cells and T* thymoma cells.

had been irradiated or treated with antimetabolic agents. X-irradiation of FcR_γ^+ B cells at 130 or 1,000 R did not affect the production of SBF at all (data not shown), and the culture supernatant of FcR_γ^+ B cells from MMC or AcM-D-treated spleen cells could also exert significant suppression on LPS responses (55–80% suppression; Table 3). On the other hand, the culture supernatant of FcR_γ^+ B cells from PurM (10 μg/ml)-treated spleen cells failed to suppress LPS responses.

These findings indicate that *de novo* protein synthesis is required for the appearance of SBF, and that DNA/RNA synthesis is not necessary for SBF production.

DISCUSSION

The immunological roles of Fc receptor (FcR) on murine lymphocytes are still controversial. It has been reported so far that immunogloublins or immune complexes binding to FcR induce an enhancing effect on immune responses in some cases (Stoner & Terres, 1960; Henry & Jerne, 1968; Pearlman, 1967; Berman & Weigle, 1978; Morgan & Weigle, 1980) while suppressing in other cases (Sinclar & Chan, 1971; Ryan & Henkart, 1976; Stockinger & Lemmel, 1978; Oberbarnscheidt & Kölsch, 1978; Morgan & Tempelis, 1978). Our studies demonstrated a possible involvement of FcR_γ^+ B cells in the regulation mechanism of immune responses by coupling immune complexes. FcR_γ^+ spleen cells from which Sephadex G-10 adherent and T cells were removed, (less than 0·5% non-specific esterase-positive cells), are suppressive for anti-DNP PFC responses and that they produce an immunoregulatory lymphokine termed SBF after stimulation of FcR_γ by immune complexes. Furthermore, EA-coupled peritoneal macrophages never release a suppressive factor such as SBF (Masuda *et al.*, 1978). Although we cannot completely exclude a possible involvement of non-adherent macrophages, it is the most probable that FcR_γ^+ B cells are SBF producers. SBF suppresses PFC responses in an antigen-non-specific but H-2 restricted manner, i.e.

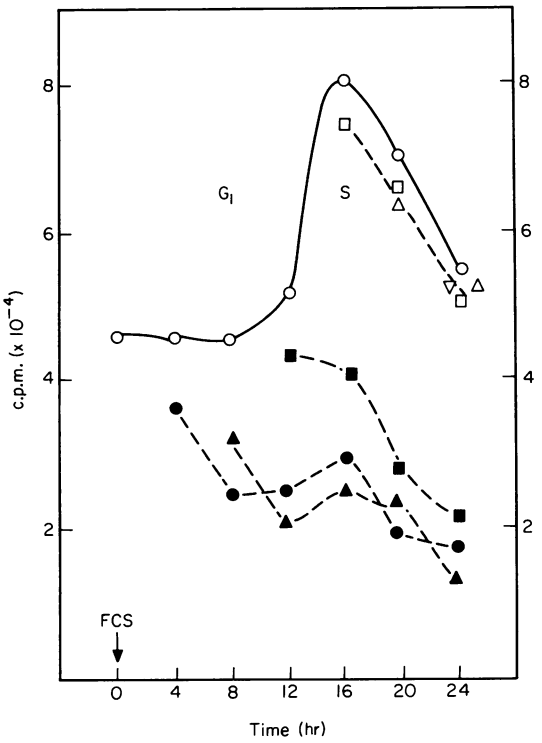

Figure 3. Cell cycle dependency on the effect of SBF. L-1210 cells were synchronized by the serum starvation method. SBF derived from BALB/c mice was added in final concentration of 5% to the cultures containing synchronized L-1210 cells at 4 (●---●), 8 (▲---▲), 12 (■---■), 16 (□---□), 20 (△---△) or 24 (▽) hr after the supplement with FCS. Cultures were pulsed with 1 μCi [^{3}H]TdR 4 hr before the harvest, and were harvested every 4 hr to measure DNA synthesis. Each symbol represents mean c.p.m. of triplicate cultures. (○) No SBF added.

concordance of H-2 haplotype between mice providing SBF and target spleen cells is necessary for SBF to perform the suppressive effect (Miyama *et al.*, 1979).

In the present studies, *de novo* synthesis of SBF is confirmed by the facts that at least 6 hr is required for the production of SBF after stimulation by immune complexes (Table 1), and that the cells treated with PurM, but not with X-ray, MMC or AcM-D, fail to release SBF (Table 3). These findings indicate that SBF is really produced by FcR_γ^+ B cells after stimulation by immune complexes, and that the release of SBF is not a simple discharge as in the case of histamin or serotonin degranulation from an intracellular store (Becker & Henson, 1973). The establishment of a SBF-producing hybridoma obtained by the

Table 3. Pharmacologic modulation of the production of SBF

Antibiotics treatment*	LPS response† c.p.m.	% Suppression§
None	3648 ± 309¶	67
Mitomycin C 50 μg/ml	4537 ± 258¶	59
5	3412 ± 704¶	69
Actinomycin D 1 μg/ml	49861 ± 249**	55
0·1	2183 ± 268¶	80
Puromycin 10 μg/ml	9988 ± 2520††	9
1	3474 ± 724¶	69
Control LPS response‡	11,030 ± 516	

* The spleen cell was treated with mitomycin C, actinomycin D or puromycin at indicated doses in the Table.

† The suppressive activity of the culture supernatant of FcR_γ^+ B cells obtained from treated spleen cells was tested by LPS responses. Mean c.p.m. ± standard error of triplicate cultures.

‡ LPS response in the absence of SBF.

§ Mean % suppression as compared with the control response.

¶ $P < 0{\cdot}001$, as compared with the control response.

** $P < 0{\cdot}01$.

†† Not significant.

fusion of FcR_γ^+ B and 3T3-4 E cells supports the presence of the gene coding SBF in FcR_γ^+ B cells which controls *de novo* synthesis of SBF, as shown in following article (Suzuki *et al.*, submitted).

SBF is suppressive for (i) PFC responses and affects precursor B cells in the induction (Fig. 1), but not effector phase, (ii) mitogen responses to LPS, but neither to Con A nor to PHA (Fig. 2), and (iii) the proliferation of tumour cells of B-cell origin, but neither of T nor of fibroblast origin *in vitro* (Table 2). Taken together, it seems possible that the suppression of PFC responses by SBF is due to the suppression of the proliferation of precursor B cells, and not to cytotoxic effects or blockade of antibody release from antibody forming cells. The possibiity that different factors are responsible for causing the different effects described above is unlikely as absorption of SBF by B-cell tumours, such as L-1210 cells, completely abrogates all the suppressive activities of SBF (Suzuki *et al.*, submitted).

It is noteworthy that SBF suppresses the proliferation of L-1210 cells *in vitro* by acting at G_1-S junction

of the cell cycle (Fig. 3). This implies that the acceptor site for SBF appears at this stage, or that the receptor might be present throughout the cell cycle, but transinduction may not occur at phases other than at the G_1-S junction. The receptor for inhibitor of DNA synthesis (IDS) is also known to appear in the late G_1 phase (Wagshal & Waksman, 1978). However, IDS and SBF are quite different, because IDS is a product of T cells stimulated with a specific antigen, PHA, Con A or allogeneic cells. Moreover, IDS suppresses not only LPS but also PHA or Con A responses and inhibits the growth of fibroblasts.

The clinical application of SBF for B-cell tumours may be attractive in the near future. In fact, we know that the administration of semipurified SBF produced by hybridoma into L-1210 bearing DBA/2 mice prolongs the survival and prevents 50% of mice used from death for 6 months (Suzuki *et al.*, submitted).

It has been already reported that, in combination experiments using various kinds of B.10 congeneic mice, the matching of the right hand side of the H-2 complex is absolutely required for the suppression of PFC responses (Miyama *et al.*, 1979). Concordance of the H-2 complex between SBF-producing mice and target B cells was also necessary for the suppression of LPS responses and the proliferation of B tumour-cell lines *in vitro* (Table 2), suggesting again that a similar or identical molecule exerts a suppressive effect against the proliferation of these B cells.

Several inflammatory or immunoregulatory lymphokines have been reported also to be produced by the stimulation of FcR of lymphocytes with immune complexes, such as leucocyte-migration inhibition factor (LIF; Neville & Lischner, 1982). Monocyte-macrophage chemotactic factor (MCF; Rocklin, Bendtzen & Greineder, 1980), tissue factor (TF; Rothberger, Zimmerman & Spiegelberg, 1977), and T cell-replacing factor ((Fc)TRF; Thoman & Weigle, 1982). All of these factors, are known to be produced by non-B lymphocytes. Immunoglobulin binding factor (IBF; Fridman *et al.*, 1976) has some similarities to SBF, because of the expression of Ia antigen and of antigen-nonspecific supression of IgM/IgG antibody responses. However, SBF is produced by FcR_γ^+ B, but not by FcR_γ^+ T cells which synthesize IBF. Moreover, SBF does not bind to IgG molecules (Miyama *et al.*, 1979). Thus, it is clear that SBF has unique properties different from any of these chemical mediators, including IBF, because of its cellular origin, biological activity, target specificity and MHC genetic restriction. To date, there is no evidence that SBF or SBF-like materials are produced by other mechanisms except for stimulation by immune complexes.

Recently, we have found suggestive evidence that SBF plays a physiological role *in vivo* to regulate immune responses (Miyama-Inaba *et al.*, 1982). The analysis for the SBF receptor on target B cells, the purification of SBF, and the characterization of anti-SBF antibody are important to understand the physiological mechanism of FcR-dependent immune regulation, in relation to immune disorders including the surveillance for the growth of B-cell tumours. This work is in progress, and the physicochemical and antigenic nature will be reported in a following article (Suzuki *et al.*, submitted).

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REFERENCES

BECKER E.L. & HENSON P.M. (1973) In vitro studies of immunologically induced secretion of mediators from cells and released phenomena. *Adv. Immunol.* **17,** 93.

BERMAN M.A. & WEIGLE W.O. (1978) B-lymphocyte activation by the Fc region of IgG. *J. exp. med.* **146,** 241.

BROOKS R.F. (1976) Regulation of the fibroblast cell cycle by serum. *Nature (Lond.)*, **260,** 248.

DICKLER H.B. (1976) Lymphocyte receptors for immunoglobulin. *Adv. Immunol.* **24,** 176.

FRIDMAN W.H., GISLER R.H., GUIMEZANES A. & NEAUPORT-SAUTES C. (1976) Suppression of *in vitro* antibody synthesis by a T cell product: its relation with the Fc receptor of activated T cells. In: *Leucocyte Membrane Determinants Regulating Immune Reactivity* (eds by V. P. Eijsvoogel, D. Roos and W. P. Zeijlemaker), p. 331. Academic Press, New York.

HENRY C. & JERENE N.K. (1968) Competition of 19S and 7S antigen receptors in the regulation of the primary immune responses. *J. exp. Med.* **128,** 133.

KÖLSCH E., OBERBARNSCHEIDT J., BRÜNER K. & HEUER J. (1980) The Fc receptor: Its role in the transmission of differentiation signals. *Immunol. Rev.* **49,** 61.

MASUDA T., MIYAMA M., KURIBAYASHI K., YODOI J., TAKABAYASHI A. & KYOIZUMI S. (1978) Immunological properties of Fc receptor on lymphocytes. 5. Suppressive immune response by Fc receptor bearing B lymphocytes. *Cell. Immunol.* **39,** 238.

MIYAMA M., YAMADA J. & MASUDA T. (1979) Immunological properties of Fc receptor on lymphocytes. 6. Characterization of suppressive B cell factor (SBF) released from Fc receptor-bearing B cells. *Cell. Immunol.* **44,** 51.

MIYAMA-INABA M., SUZUKI T., PAKU Y-H. & MASUDA T. (1982) Feedback regulation of immune responses by immune complexes; possible involvement of a suppressive lymphokine by FcR_γ-bearing B cell. *J. Immunol.* **128,** 882.

MORGAN E.L. & TEMPELIS C.H. (1978) The requirement for the Fc portion of antibody in antigen–antibody complex-mediated suppression. *J. Immunol.* **120,** 1669.

MORGAN E.L. & WEIGLE W.O. (1980) Regulation of Fc fragment-induced murine spleen cell proliferation. *J. exp. Med.* **151,** 1.

NEVILLE M.E. & LISCHNER H.W. (1982) Activation of Fc receptor-bearing lymphocytes by immune complexes. 1. Stimulation of lymphokine production by non-adherent human peripheral blood lymphocytes. *J. Immunol.* **128,** 1063.

OBERBARNSCHEIDT J. & KÖLSCH E. (1978) Direct blockade of antigen-reactive B lymphocytes by immune complexes. An 'off' signal for precursors of IgM-producing cells provided by the linkage of antigen- and Fc-receptors. *Immunology.* **35,** 151.

PEARLMAN D.S. (1967) The influence of antibody on immunologic responses. 1. The effect on the response to particulate antigen in the rabbit. *J. exp. med.* **126,** 127.

ROCKLIN R.E., BENDTZEN K. & GREINEDER D. (1980) Mediators of immunity: lymphokines and monokines. *Adv. Immunol.* **29,** 55.

ROTHBERGER H.T., ZIMMERMAN T.S. & SPIEGELBERG H.S. (1977) Leukocyte procoagulant activity. Enhancement of production *in vitro* by IgG and antigen-antibody complexes. *J. clin. Invest.* **59,** 549.

RYAN I.L. & HENKART P.A. (1976) Inhibition of B lymphocytes activation by interaction with Fc receptors. *Immunol. Comm.* **5,** 455.

SCHIMPL A. & WECKER E. (1979) Lymphokines in nonspecific T cell–B cell cooperation. In: *Biology of the lymphokines* (eds S. Cohen, E. Pick and J. J. Oppenheim) p. 369. Academic Press, New York.

SINCLAR N.S.StC. & CHAN P.L. (1971) Regulation of the immune response. IV. The role of the Fc-fragment in feedback inhibition by antibody. *Adv exp. Med. Biol.* **12,** 609.

STOCKINGER B. & LEMMEL E. (1978) Fc receptor dependency of antibody mediated feedback regulation: on the mechanism of inhibition. *Cell. Immunol.* **40,** 395.

STONER R.O. & TERRES G. (1960) Enhanced antitoxin responses in irradiated mice elicited by complexes of tetanus toxoid and specific antibody. *J. Immunol.* **91,** 761.

THEOFILOPOULOS A.N. & DIXON F.J. (1979) The biology and detection of immune complexes. *Adv. Immunol.* **28,** 89.

THOMAN M.L. & WEIGLE W.O. (1982) Preliminary chemical and biologic characterization of (Fc)TRF: an Fc fragment-induced T-cell replacing factor. *J. Immunol.* **128,** 590.

UHR J.W. & MÖLLER G. (1968) Regulatory effect of antibody on the immune response. *Adv. Immunol.* **8,** 81.

WAGSHAL A.B. & WAKSMAN B.H. (1978) Regulatory substances produced by lymphocytes. VIII. Cell cycle specificity of inhibitor of DNA synthesis (IDS) action in lymphocytes. *J. Immunol.* **121,** 966.