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The effects of new T cell line derived lymphokines on B cell activation

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ABSTRAK

Sofia Mubarika Haryana – *Pengaruh limfokin yang berasal dari T cell line baru terhadap aktivasi sel B*

Telah diketahui bahwa untuk aktivasi limfosit B diperlukan faktor pertumbuhan yang disebut limfokin atau sitokin. Banyak bukti menunjukkan bahwa limfosit T yang teraktivasi menghasilkan substansi yang dapat memacu proliferasi atau diferensiasi limfosit B. Dewasa ini telah dapat diidentifikasi berbagai limfokin yang disebut sebagai interleukin (IL) seperti IL-2, IL-3, IL-4, IL-5, IL-6 dan IL-7 yang ikut berperan dalam aktivasi sel B. Namun, mekanisme aktivasi sel B oleh limfokin sampai sekarang masih terus berkembang dan dipelajari. Untuk dapat lebih memahami aktivasi sel B, dalam penelitian ini telah dilakukan pembuatan suatu T *cell line*, dan telah dikarakterisasi, yakni menunjukkan fenotipe Thy1$^+$, Lyt1$^+$2$^+$, sIg$^-$. Dalam penelitian ini berbagai aktivator poliklonal lipopolysaccharide (LPS), ConA, dextran sulfat dipakai untuk memacu T *cell line* menghasilkan limfokin. Aktivitas limfokin pada supernatannya kemudian dianalisis dengan menggunakan *blastogenesis assay* dengan ^{3}H-thymidine dan *hemolytic plaque assay*.

Hasil penelitian menunjukkan bahwa T *cell line* menghasilkan faktor pertumbuhan yang memacu proliferasi sel B (B cell growth factor II = BCGF II atau IL-5) dan faktor yang memacu sel B untuk berdiferensiasi (B cell differentiating factor = BCDF atau IL- 6) dan menghasilkan IgM dan IgG.

Key words : blastogenesis assay – hemolitic plaque assay – B cell activation – T cell line – lymphokines

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INTRODUCTION

The immune system consists of a network of cells that interact through direct contact and soluble mediators.[1,2] The normal cellular components of the immune system are the macrophages and lymphocytes.[3] Macrophages have a variety of functions, i.e., to process and present antigens to lymphocytes[4] and determine which T or B cell will be induced to a functional state.[5] Macrophages also secrete biologically active mediators which regulate both T and B cell response.[6] Both T and B cells have antigen specific receptors on the surface of every immunocompetent cells.[7]

The regulation of immune response is a function of T lymphocytes. It has been generally accepted that the regulatory cells are either T helper cells (Th) which govern a positive regulatory effect[8] or T supressor cells (Ts) which exert a negative regulatory effect on both T and B cells.[9] Several authors reported that lymphokines produced by B cells also influence antibody production *in vitro*.[10] The initiation of B cells to proliferate, differentiate and secrete is less understood than that of T cells, and specific growth factors for B cells have been identified.[11] Active cooperation is required between antigen-specific T helper cells, B cells and macrophages for the generation of antibody responses to antigen. The interaction between Th cells and macrophages is genetically restricted by products of the major histocompatibility complex (MHC).[12]

Various factors derived from T cells have been shown to mediate proliferation and differentiation of B cells. Many kinds of lymphokines which induce proliferation and differentiation of B cells have been documented and indicate that receptors for different lymphokines are expressed at various

stage of B cell maturation and also the development of B cells proceed a series of lymphokines responsive differentiation stage.[13,14]

This study was aimed at investigating the stimulatory effect of lymphokines produced by a new T cell line in B cell to finally produce Ig.

MATERIALS AND METHODS

Animals

Mice used in this study were CBA/N, C57BL/10J, C3H/HeN and CDFl (BALB/c x DBA/2J) F1 mice. Mice were maintained on diet 86 pellets and water. Offsprings were weaned at 4 weeks of age and used between 6 and 12 weeks of age.

Cell lines

The cell line CTB (an IL-2 dependent T cell line), FDCP-2 (an IL- 3 dependent mast cell line), I cell (an IL-2 dependent T cell line), BCL-1 (B cell lymphoma line) were used in this study. These cells were maintained in suspension culture. BCL-1 cell were maintained *in vivo* by passaging cells intraperitoneally in CDFl mice.

Chemicals

Stock solution of Concanavalin A (ConA) (Calbiochem Behring Corp., La Jolla, California, USA), lipopolysaccharide (LPS) (*Escherichia coli* K 235) (DIFCO, Detroit, Michigan, USA) and Dextran sulphate ($DxSO_4$) (sodium salt, MW 500.000, Sigma Chemical Co., St Louis, Mo, USA) were prepared in 0.9% saline, dispersed in a small volume and stored at 4°C until used. Phytohemagglutinin (PHA) (Burroughs Wellcome Laboratories, Research Triangle, N.C.) was received as powder which was then reconstituted to 5 ml with 0.9% saline. Chromic chloride ($CrCl_3.6H_2O$) Merck was used to couple proteins to red blood cells.

Radiochemicals

Radioactive thymidine was obtained from New England Nuclear, with specific activities of 2 Ci/mol. An amount of 0.5 l were added to each culture. The labelling period for IL-1 was 16 hours, while for IL-2, Il-3, granulocyte macrophage colony stimulating factor (GM-CSF), I cell factor (ICF) and BCL-1 assay was 6 hours.

Solutions and media

a. HEPES-buffer balanced salt solution was sterilized by filtration as described and stored at 4°C.[15]
b. Rat Factor Conditioned Medium (RAFT)
   IL-2 conditioned medium was prepared by stimulating rat splenocytes at a density of 10^7 cell per ml with 4 g/ml of ConA, then collected after 24 hours.
c. WEHI-3 Conditioned Medium (WEHI-3 CM)
   WEHI-3 CM was prepared by culturing WEHI-3 cells in medium RPMI-1640, supplemented with fetal calf serum, glutamin, gentamycin and 2-mercaptoethanol at density of 1 $\times$ 10^6 cells/ml for 48 hours. Supernatant was harvested, centrifuged, filtered and stored at -20°C.
d. Rose Park Memorial Institute (RPMI) 1640
   RPMI 1640 was obtained in dry powder from GIBCO, Cleveland, Ohio, USA. It was dissolved in milli-Q glass distilled water, added with sodium bicarbonate (2 g/litre) and pH adjusted to 7.2 concentrated HCl. The solution was filtered using 0.22 µm millipore and stored at 4°C.
e. Fetal Calf Serum (FCS) was obtained from GIBCO, N.Z. Ltd., Auckland, stored at -15°C. Before use FCS was heat inactivated in a 56°C water bath for 30 minutes.
f. Culture medium for maintaining I cells and CTB cells was supplemented with 5% RAFT. For FDCP-2 the medium was supplemented with 10% WEHI-3 CM.
g. Scintillation fluid was prepared by adding PPO (2,5-diphenyl oxide) 5 gr, POPOP (p-bis-(2-(phenyloxazolyl)) benzine) 100 mg and toluen to 1000 ml.

Preparation of cell suspension

Mice were killed by cervical dislocation and the spleen, thymus and femur were removed aseptically. The cells were teased gently into culture medium in petri dish (Falcon, USA) using

forceps or syringe 25 G needle. Clumps of cells and debris were removed. Cells were counted in counting fluid (6% acetic acid, 0.1% methyl violet in saline) in a hemocytometer under phase contrast microscope.

Derivation of I cell line

The T cell line which was termed I cells used in this study were originally derived in the following way. CBA/N mice were injected with 0.2 ml T24-31.7 ascitic fluid, and sacrificed after 4 days by cervical dislocation. Spleens were removed and single cell suspension was prepared at density 5×10^6 cell. Cells were cultured in medium supplemented with IL-3 and 10 g/ml LPS with irradiated syngenic cells (3000R) as fillers at a density of 2×10^7 cells/ml. After 12 - 16 weeks large growing cells emerged from the culture. These cells were cloned and characterized.

Biological assay

a. Mitogen assay
Mitogen assay was used to detect functional T and B lymphocytes population in *in vitro* cultures. The sterile suspension of cells in a density of 5×10^6 for thymocytes or 2×10^6 cells/ml for the others were used. 0.2 ml of those cells were distributed into wells and then mitogen were added. The concentration of ConA varied from 0.01 to 20 g/ml. $DxSO_4$ varied between 0.01 and 500 g/ml. The culture were set in duplicate and kept in CO_2 incubator (5% CO_2, 95% air at 37°C).

b. Kinetics of factor production
The kinetics of factor production was examined by using different condition media to stimulate I cells to produce ICF. Macrocultures were set up in a Costar well (Nunc, Nuclone, Denmark) containing 5×10^5 I cells/ml. The conditioned media RAFT, WEHI-3 CM, mitogenic agents, PHA, and ConA were tested for their effects on factors produced. At various times (day 0,1,2,3), supernatant from the various macrocultures was harvested and tested for mitogenic activity on BCL-1 tumor cells and anti-Thy1 depleted spleen cells.

b1. IL-2: Into 96 well flat bottom plates, samples were serially diluted in 100 l of complete medium. As positive control 1/10 dilution of RAFT and as negative controls only complete medium was used. CTB or CTLL at a density of 4×10^4 cells/ml, were used as much as 100 µl/well. The incubation time was 20-24 hours, followed by radiolabelling for 4 hours with 0.2 µCi (^{3}H)-thymidine.

b2. IL-3: As positive control MC-9, WEHI-3 or 5A4 supernatants, and as negative control only complete medium was used. FDCP-2 cells at 5×10^4/ml were distributed into 100 µl/well in the plate. The incubation and radiolabelling time was the same as b1.

b3. GM-CSF: Positive controls MC9, WEHI-3 or 5A4 supernatants and as negative control, the complete medium was used. Bone marrow cells at density of 1×10^6 cells/ml, were used as much as 100 µl/well. Samples were incubated and radiolabelled.

b4. I Cell-Derived Factor (ICF): The positive control was I cell supernatant, and as negative control, the normal medium only were used. Anti-Thy-1 depleted spleen cells at density of 10^6 cells/ml, and as much as 100 µl/well were added. Cultures were incubated and radiolabelled.

b5. Cell growth Assay (BCL-1)
The positive control was LPS and as negative control, the complete medium were used. BCL-1 cells grown *in vivo* were used. The spleen was excised from tumor-bearing CDFI mice, minced through a wire gauze and washed in medium. Cells were resuspended with Percoll mix (3 ml of Percoll solution + 4 ml of 2 × Balanee Salt Solution (BSS)) and centrifuged at 2500 rpm for 25 minutes in 4°C. The viable cells remained on the top of the Percoll mix and the dead cells sedimented to the bottom of the tubes. The viable cells were adjusted to 5×10^5 cells/ml, and 100 µl of cells suspension were added. Cultures were incubated and labelled.

Cell harvesting

All of the assays were harvested using an automated sample harvestor and glass fibre filter paper GFIA. The papers were dried in oven for 60 minutes. The paper were placed in scintillation vials and 2 ml of scintillation liquid was added to each vial.

Anti-Thy-1 treatment *in vivo*

Two hundred 1 of T24-31.7 ascitic fluid was injected intraperitoneally into CDF-1 mice. Spleen was removed after 4-7 days of injection, and refered to as anti-Thy-1 depleted spleen cells.

Hemolytic Plaque assay

The hemolytic plaque method allows to visualize a small amount of lytic antibody (10^3 to 10^6 molecules) released in the vicinity of a single lymphocyte. Lymphocyte and a dense suspension of indicator red blood cells were mixed and distributed in a thin layer as described by Henry.[16] The medium of 1 × BSS 5% FCS. 0.5 ml of packed sheep red blood cell (SRBC) was put in a tube after washing with Alsevier solution and BSS. 0.5 ml of rabbit anti mouse Ig (RAM-Ig) coupling reagent, and 0.5 ml of $CrCl_3$ solution (1/100 in saline, Merck) was added. The tubes were vortexed for 5 minutes and washed in saline. Sensitized lymphocytes were washed once with BSS, and 100 1 of this cell suspension was transferred and diluted through 100 1 medium in 1:100 and 1:1000. 100 1 of 0.5% RAM-Ig coupling sheep erythrocytes in BSS was added to all wells. The tray containing lymphocytes was centrifuged 5 minutes at 250 rpm. The tray was incubated at 37°C for 90 minutes. One drop/well of 1:10 RAM-Ig developer was added, and incubated for 60 minutes. A complement was added in BSS 1 drop/well and incubated for 60 minutes. The plaques were counted under light microscope using 10x magnification.[16] Positive plaques showed a clear area with one cell or more in the middle of this area. False positivity could have been due to air bubbles, lumps of cells, tissue debris, small bacteria colony or long incubation more than 6 hours.[17]

RESULTS

Kinetics of factor production

FIGURE 1 shows that stimulatory activity for BCL-1 cells exists in all of the conditioned media used. The highest activity was seem on day 2, exept in ConA conditioned media. On day 3, the stimulation activity could not be detected.

The mitogenic activity of I cell factor was assayed using anti-Thy-1 depleted spleen cell cultures. FIGURE 2 shows that all of the conditioned media tested containing factors that stimulated anti-Thy-1 depleted spleen cells to proliferate. On day 1, 2, and 3, this factor activity was detected, but not on day 0.

Kinetics of IL-3 production

According to Ihle *et al.*,[1,18] IL-3 induces the expression of an enzyme 20 α-steroid dehydrogenase (20 α-SDH) in culture of nude splenic lymphocytes and bone marrow cells. T cell mitogens, such as ConA and PHA, induced IL-3 activity in lymphocyte culture, whereas B cell mitogen had no effect. IL-3 was assayed using FDCP-2 proliferation assay. FIGURE 3A and 3B showed that I cell-conditioned media prepared using 2 and 10 µg ConA contained high IL-3 titres. The other conditioned medium did not show such an activity. Thus, ConA appeared to induce the secretion of IL-3 from I cells.

Kinetics of IL-2 production

A variety of T cell lines produced IL-2 after mitogen stimulation.[19] This experiment was carried out to determine whether I cells produced IL-2. The data presented in FIGURE 4 revealed no stimulating activity in any of the I cell supernatants. Thus, I cells did not produce IL-2.

Effect of mitogen on BCL-1 cells

The effect of lymphocyte mitogens on the response of BCL-1 cells to ICF was studied. The B cell mitogen LPS and $DxSO_4$ and the mitogen ConA were used. The data presented in FIGURE 5A revealed that ICF had synergistic effect with $DxSO_4$ (10 µg/ml) in the stimulation of BCL-1

cells. However, when the concentration of $DxSO_4$ was increased to 500 µg/ml, the synergistic effect was not seen, but there was inhibition of the BCL-1 response.

In assaying the synergistic effects, $DxSO_4$ and LPS were also added together in different concentrations and assayed for B cell proliferation using BCL-1 cells. Interestingly, the data presented in FIGURE 5B showed that $DxSO_4$ and LPS had synergistic effect on the stimulation of BCL-1 cells. In contrast, the data presented in FIGURE 5C revealed that ConA had an inhibitory effect on ICF.

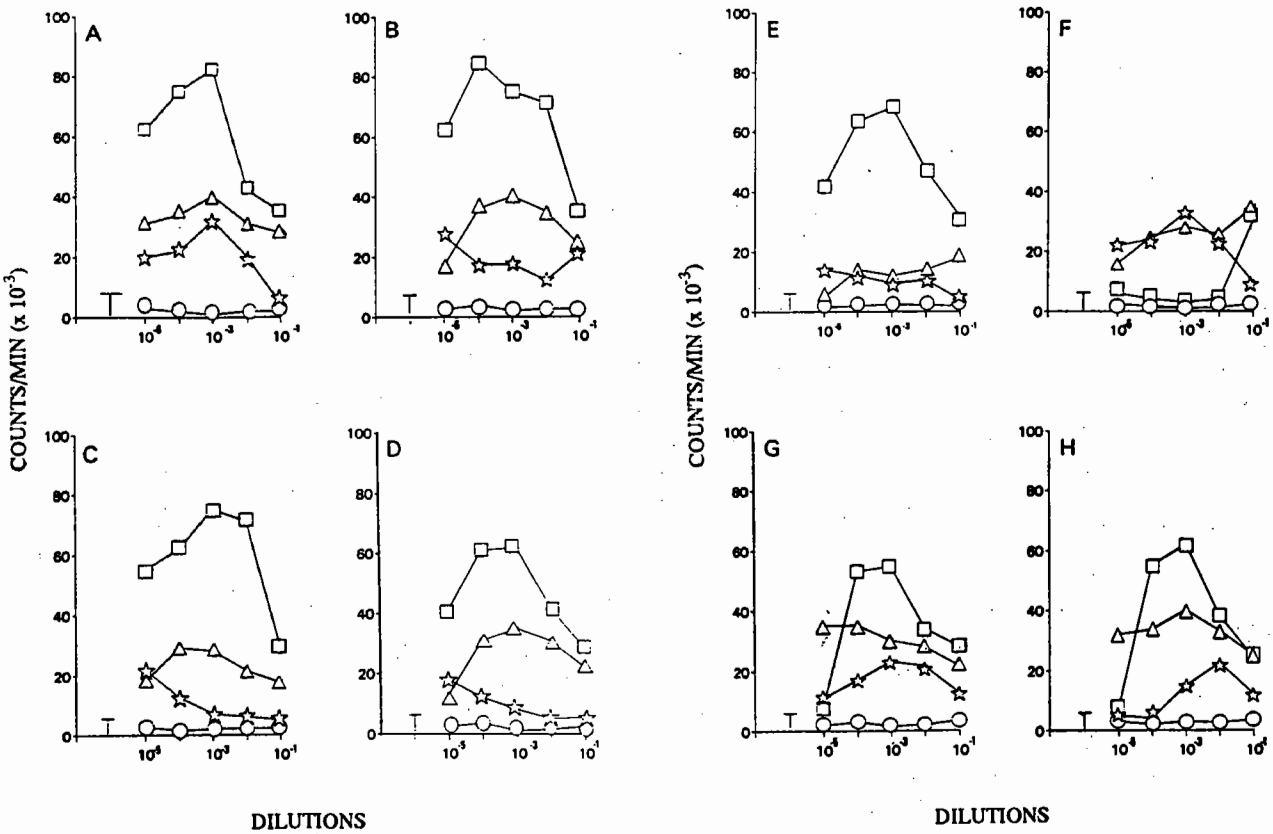

FIGURE 1. Kinetics of ICF production on BCL-1 proliferation assay

I cells at the density of 5 x 10^5 cells/ml were cultured with: A) medium only; B). 5% RAFT; C) 20% WEHI-3 CM; D) 1% PHA. On day 0 (Δ), day 1 (☆), and day 2 (□), and day 3 (○), 5 x 10^4 BCL-1 cells were incubated with supernatant and titrated into these cultures. [3H]-TdR was added for these last 6 hours of a 72 hour culture period. I cells at the density of 5 x 10^5 cells/ml were cultured with: E) 5% PHA; F) 2 µg ConA; G) 5 µg ConA; H) 10 µg ConA. On day 0 (Δ), day 1 (☆), and day 2 (□), and day 3 (○), 5 x 10^4 BCL-1 cells were incubated with supernatant from the culture and titrated into these cultures. (^{3}H)-TdR was added for the last 6 hours of a 72 hours culture period.

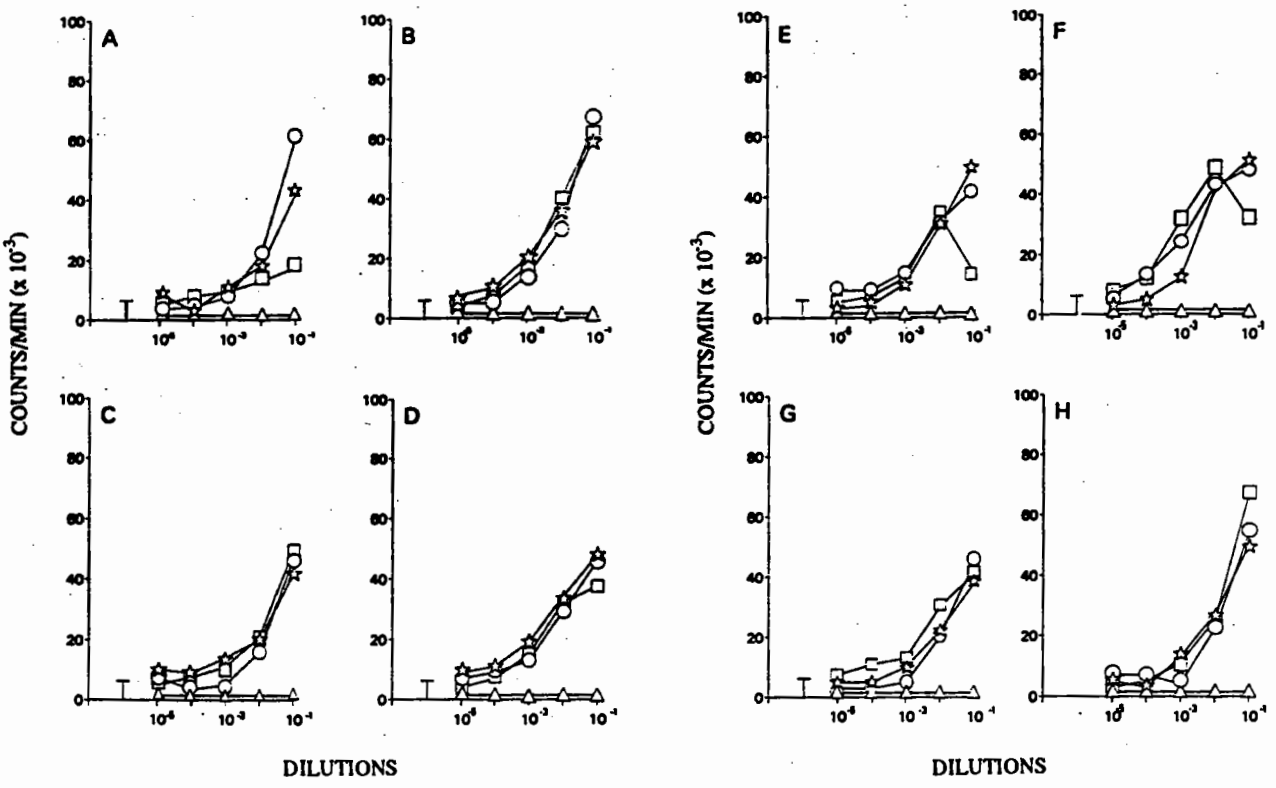

FIGURE 2. Kinetics of ICF production and its effect on anti-Thy-1 depleted spleen cells

Macrocultures of I cell at the density of 5 x 10^5 cells/ml were set up using different conditioned media: A) medium only; B) 5% RAFT; C) 20% WEHI-3 CM; D) 1% PHA. At various time, day 0 (Δ), day 1 (☆), day 2 (❑), day 3 (○), 5 x 10^5 anti-Thy-1 depleted spleen cells were incubated with titrated supernatant. [^{3}H]- TdR was added for the last 6 hours of a 72 hour culture. Macrocultures of I cell at the density of 5 x 10^5 cells/ml were set up using different conditioned media: E) 5% PHA; F) 2 µg/ml ConA; G) 5 µg/ml ConA; H) 10 µg/ConA. At various time, day 0 (Δ), day 1 (☆), day 2 (❑), day 3 (○), 5 x 10^5 anti-Thy-1 depleted spleen cells were incubated with titrated supernatant and cultures. [^{3}H]-TdR was added for the last 6 hours of a 72 hour culture.

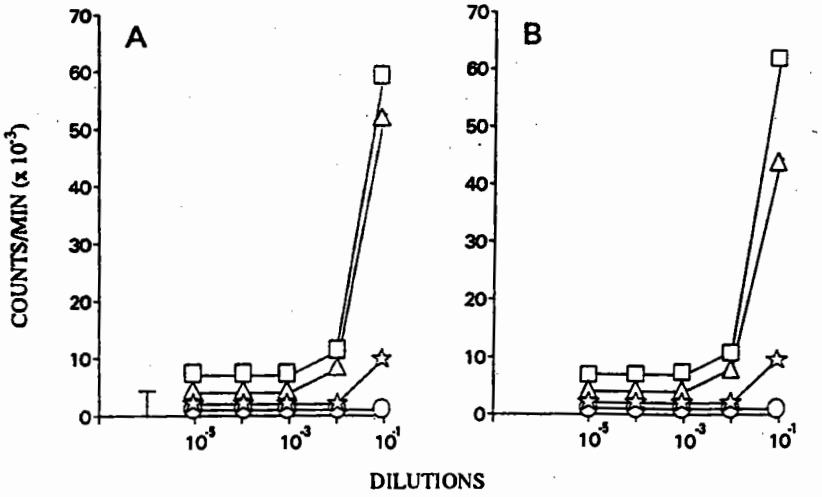

FIGURE 3. Kinetics of IL-3 production

Macroculture of cells at the density of 5 x 10^5 cells/ml were set up with different kinds of conditioned media: A) 2 µg ConA; B) 10 µg ConA. On day 0 (Δ), day 1 (☆), day 2 (❑), and day 3 (○), using FDCP-2 cells (Section 2.4.8 Cii), factors were titrated through the well. The final dilution was 1/10, $1/10^2$, $1/10^3$, $1/10^4$, $1/10^5$. The activity of the factors was assessed by thymidine radioactive incorporation.

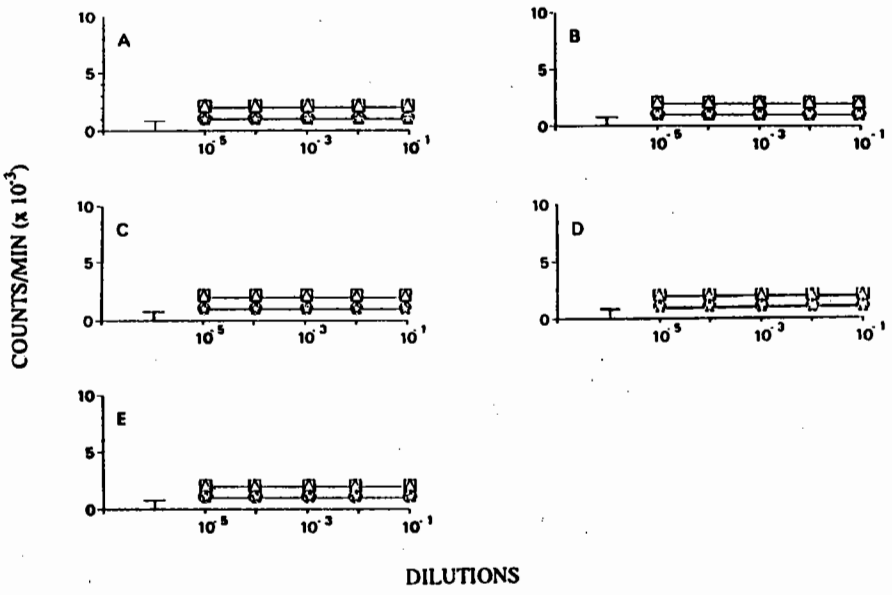

FIGURE 4. Kinetics of IL-2 production

Macroculture of cells at the density of 5 x 10^5 cells/µl were set up using different kinds of conditioned medium: CTB cells were used at a density of 4 x 10^4 cells/ml in microculture (using 100 l/well). Supernatant was tested through serial ten-fold dilution. The final dilution were 1/10, 1/10^2, 1/10^3, 1/10^4, and 1/10^5. The mitogenicity of factors were assessed by thymidine radioactive incorporation.

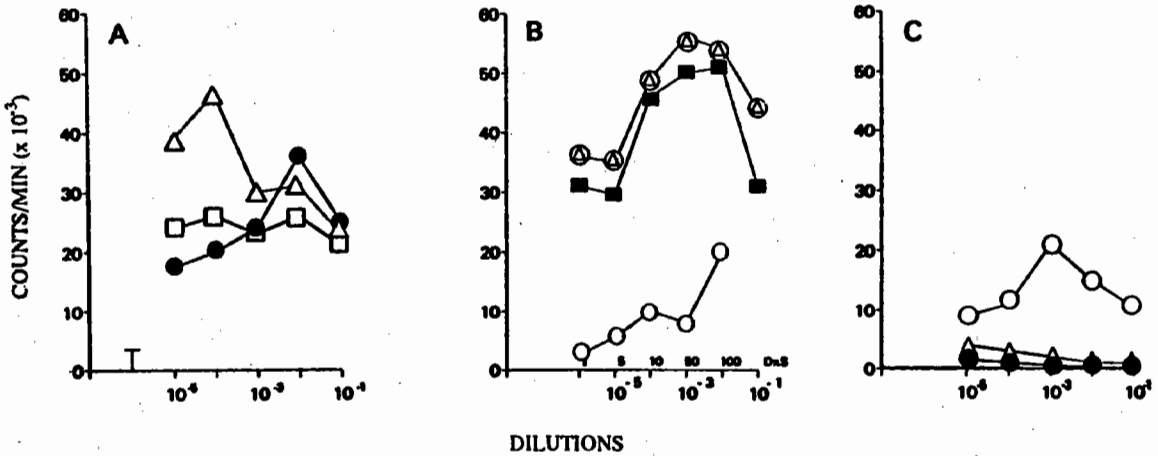

FIGURE 5. Effect of $DxSO_4$, LPS, and ConA on BCL-1 cells

A: $DxSO_4$ at the concentration of 10 µg/ml and 500 µg/ml were used as B cell mitogen. ICF was titrated through and the final dilutions were 1/10, 1/10^2, 1/10^3, 1/10^4, and 1/10^5. BCL-1 cells at a density of 5 x 10^4 cells/ml were added. After 72 hours incubation, cultures were radioactively labelled with titrated thymidine. ● = ICF alone; Δ = $DxSO_4$ g + ICF; ❑= $DxSO_4$ 500 µg + ICF B: The microcultures were set up using, 1, 5, 10, 50, and 100 µg/ml of $DxSO_4$. LPS was added using 5 g and 10 µg/ml. BCL-1 cells at a density 5 x 10^4 cells/ml were added. after 72 hours incubation, cultures were radioactively labelled with titrated thymidine. ○ = $DxSO_4$; ◬ = $DxSO_4$ 500 µg + LPS µg/ml; ■= $DxSO_4$ µg + LPS 5 µg/ml. C: The microcultures were set up using, 0.5, 1, 5, and 10 of ConA. I factor was titrated through and the final dilutions were ty 1/10, 1/10^2, 1/10^3, 1/10^4, and 1/10^5. BCL-1 cells at a density of 5 x 10^4 cells/ml were added. The mitogenicity respons of culture was assessed by thymidine radioactive incorporation. Δ = ConA; ●= ConA + ICF; ○ = ICF

Effect of ICF on other cells

The effects of ICF were studied on thymocytes, spleen and bone marrow cells. FIGURE 6 represented the effect of ICF on spleen cells. The result indicated that ICF did stimulate bone marrow, thymocytes and spleen cells, but the growth promoting activity in these cells did not show much stimulation.

The formation of Ig producing cells

The effect of ICF on BCL-1 cells showed that the pellet containing factors which stimulate B cells to differentiate into Ig-secreting cells (TABLE 1). The data presented in TABLE 2 showed that ICF did not stimulate BCL-1 cells to differentiate into antibody-producing cells.

The kinetics of the differentiation of anti-Thy-1 depleted spleen cells into immunoglobulin producing cells was examined. The results presented in TABLE 3 showed that I cell supernatant stimulated anti-Thy-1 depleted spleen cell to differentiate into immunoglobulin-producing cells. More plaques could be detected on day 4 than on days 2 or 3.

DISCUSSION

The I cell had phenotype of $Thy1^+$, $Lyt1^+2^+$ and sIg^-. Fifty percent of I cells were $Lyt2^+$. The precursor of T cells had surface phenotype of $Thy1^+$, $Lyt1^+2^+3^+$. About 40% of peripheral blood T cells displayed Lyt1+. Including in this population were T cells that helped the differentiation of B cells into Ig secreting plasma cells, T cells that helped in the development of cytotoxic cells, and T cells that were effectors for delayed hypersensitivity.[20] $Lyt2^+,3^+$ representing approximately 20% o f T c ells, contained cyto-

TABLE 1. Effect of ICF-SN and pellet on B cell response tested by reverse plaque assay

	Number of PCF/culture
No factor	60
Pellet of ICF-SN	23,4966
Supernatant	3,250
Staring material	26,100

TABLE 2. Reverse plaque assay of ICF-SN in BCL-1 cells

	Number of PFC/Cultures		
BCL-1 + ICF	1,000	2,000	1,000
BCL-1 + ICF + LPS	–	4,000	–
BCL-1	–	–	1,000
Spleen cells + LPS	300,00 0	1,900,000	300,000

TABLE 3. The Kinetics of immunoglobulin production stimulated by ICF

	Direct PFC/10^6 Cells	Indirect PFC/10^6 Cells
No ICF	13	92
Day 2	80	93
Day 3	160	228
Day 4	224	410

Legend to Table 3.

The assay medium used was supplemented with 2ME, 5% FCS, PSG, non-essential amino acid and sodium pyruvate. B cell specific erythrocytes were prepared by priming with 0.2 ml 5% SE and 0.2 ml anti-Thy-1 antibody intraperitoneally and used on days 4 - 7 after priming

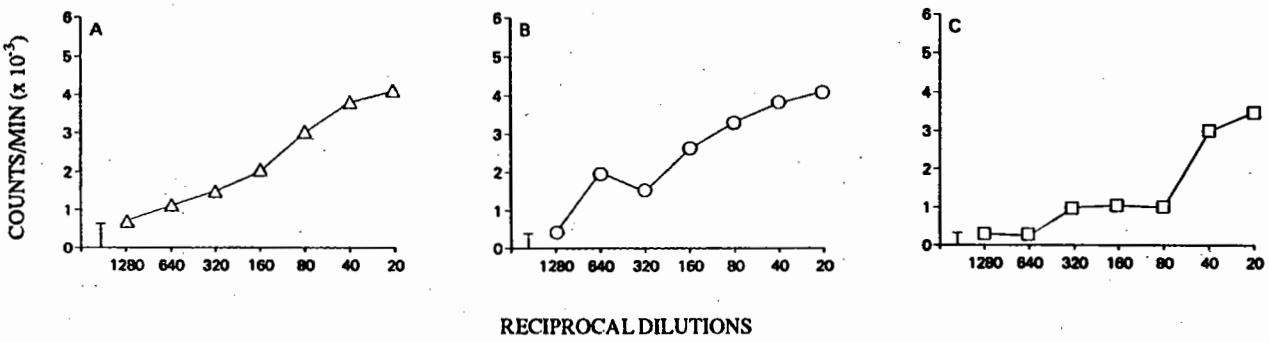

FIGURE 6: Effect of ICF on other cells

Three microcultures were set up containing: A. Δ = bone marrow cell 10^6/ml; B. ○ = thymocytes; and C. □ = spleen cells 2 x 10^6 cells/ml. Samples were titrated through 100 µl complete medium. after the incubation time (bone marrow assay: 72 hours, thymocyte assay 48 hours, spleen assay 72 hours), cultures were radioactively labelled with [^{3}H]-TdR for 6 hours.

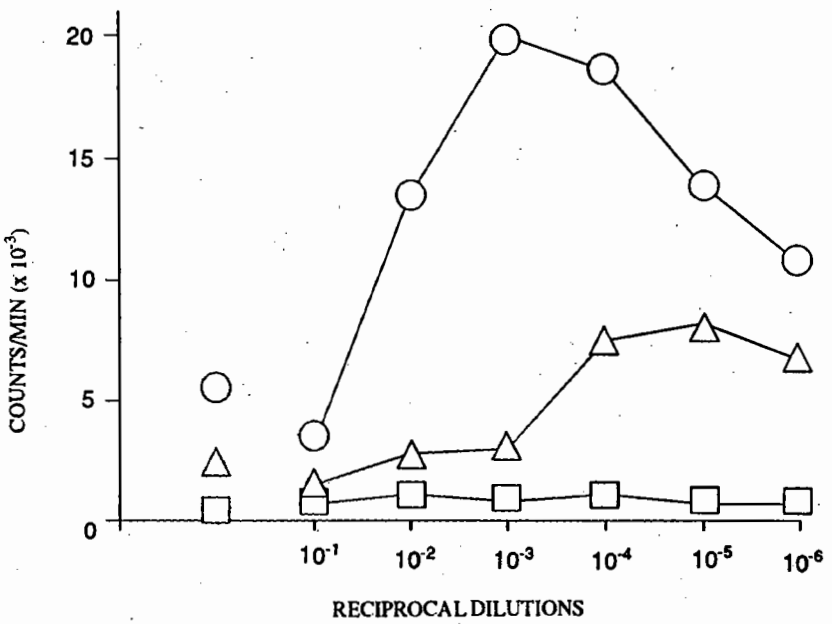

FIGURE 7. Effect of fresh vs. frozen BCL-1 cells

Microcultures were set up containing 5 x 10^4 cells/ml fresh BCL-1 cells (○), frozen BCL-1 cells less than 2 week old (Δ), frozen BCL-1 cells more than 3 month old (□). Sample of ICF were titrated through 100 l complete medium. After 72 hours incubation, cultures were pulsed with [^{3}H]-TdR for 6 hours. Negative controls used medium and positive controls used LPS.

toxic T cells and supressor T cells.

The result of this study suggested that I cells were the precursor of Lyt1$^+$ cells. Lyt1$^+$ cells were responsible for most functions associated with T cell help. This T cell help was operationally defined by the cooperation of T cells with either B lymphocytes[13] or with other T cells (which differentiate into cytotoxic effectors).[21] Lyt1$^+$ cells were also responsible for the production of T cell replacing factor (TRF) which could be substituted for Th cells in assay system.[22] Lyt1$^+$ cells also responsible for the proliferation

among T cells in which the antigen for T cell activation as soluble protein, particularly the allogeneic MHC antigen and mitogen.[23] Delayed type hypersensitivity and graft versus host reaction were also depending upon $Lyt1^+$ cells. $Lyt1^+$ cells produce IL-2, CFS, interferon gamma (IFN-), macrophage activating factors (MAF), migration inhibition factor (MIF) and a variety of B cell stimulating factors.[2]

The kinetics of factor produced showed that all the tested conditioned media yielded good stimulation, indicating that I cells produced some factors that stimulate the growth of B cells. BCL-1 cells bearing surface phenotype of $IgMR^+$, CR^- and marginally $sIgD^+$,[24] appeared to be monoclonal.[25] Thus, in this proliferation assay, no contaminating cells affected the results.

The existence of IL-2 demonstrated that I cell supernatant did not stimulate CTB to proliferate, indicating I cell did not produce IL-2.[2] This cell population might be grouped as Th2 which did not produce IL-2 but IL-5. As showed by Wegmann *et al.*[27] that subset of Th1 produced IL-2, IFN- and TH2 produced IL-4, IL-5 and IL-10.[27] The effect of the I cell supernatant on anti-Thy-1 depleted spleen cell demonstrated that all conditioned media stimulated the cells. However, the existence of other factors produced by other cells in the culture such as macrophages which produced IL-1, B cell growth factor and IFN- should be excluded. Le Gros showed that sIg^+ cells and macrophages were not affected by anti-Thy-1 antibody treatment.[27] Thus, other cells in anti-Thy-1 depleted spleen cells culture after stimulation with mitogen produced lymphokines as showed in this study. Many B cell mitogens such as $DxSO_4$, LPS, ConA have been used to see the synergistic effect of ICF on the BCL-1 proliferation response. $DxSO_4$ is a polyanion which acts as polyclonal activators of B lymphocytes, whereas LPS is able to activate small, resting B lymphocytes to proliferate and differentiate.[28,29] Moreover, $DxSO_4$ and LPS seemed to activate distinct B lymphocyte subpopulation, to proliferate and secrete antibody. ConA, as a T cell mitogenic agent, was able to activate B cell to enter the cell cycle, but it was unable to induce B cell to proliferate.[30,31] This study showed that ICF synergized with $DxSO_4$, even ICF could stimulate BCL-1 without costimulation. $DxSO_4$ itself at high concentration could stimulate B cell but not at low concentration. To differentiate whether this T cell line produce BCGF-I or BCGF-II, LPS and $DxSO_4$ were used. The results indicated that T cell produced BCGF-II or IL-5. It has been shown that $DxSO_4$ and LPS had no synergistic effect on the stimulation of BCGF-I as showed by Shimizu *et al.*[32] Thus, this study confirmed that the T cell line produces BCGF-II or IL-5. ConA and ICF showed an inhibitory effect on BCL-1 cell. The process of inhibition was posibly due to massive cross-linking of glycoprotein to ConA on the lymphocyte surface as previously reported.[33]

It was shown that ICF did not stimulate thymocytes, but slightly affected the growth of bone marrow cells. This presumably caused by other factor such as GM-CSF produced by other cell in the culture.

The plaque forming assay showed that I cell supernatant stimulated many plaques. The reverse plaque assay using BCL-1 produced very little plaque formation when compared to normal spleen cells cultured with LPS. The result indicated that T cell line produced BCDF to stimulate B cell to produce IgM and IgG. The result also showed that I cell factor stimulated anti-Thy-1 depleted spleen cells to differentiate into IgM and IgG-producing cells, indicating that these B cells expressed receptors to BCDF. Nakamishi *et al.*[34] showed that BCDF receptor was required for the differentiation of B cells into Ig producing cells. BCL-1 was unresponsive to I cell supernatant presumably due to the lack of BCDF receptors on BCL-1 tumor cells, as demonstrated by Knapp.[25]

CONCLUSION

The I cell supernatant contained BCGF-II or IL-5 and B cell differentiation factor or IL-6 to stimulate B cell to proliferate and produce IgM and IgG.

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