Immunology 1987 **60** 51–55

Enhancement of the B-cell response to *Staphylococcus aureus* Cowan strain 1 by natural human gamma interferon

N. AOKI & Y. OHNO *Department of Medicine, Kinki University School of Medicine, Sayama, Minamikawachi, Osaka, Japan*

Accepted for publication 8 September 1986

SUMMARY

The effects of interferon (IFN) on the B-cell response to *Staphylococcus aureus* Cowan strain 1 (SAC) were studied comparatively with natural human IFN-α, IFN-β and IFN-γ, employing equal units of their anti-viral activity. First, the response was investigated in peripheral mononuclear cells obtained from healthy individuals, and next, confirmed in cultures employing B-cell enriched populations derived from tonsils obtained at tonsillectomy from patients with chronic tonsillitis. B cells were purified by rosetting out T cells with sheep red cells followed by the removal of adherent cells on a plastic surface. The results show that the SAC-stimulated lymphoproliferative response was enhanced in the presence of IFN-γ in a dose-related manner, at concentrations ranging from 10 to 1000 IU/ml, both in peripheral mononuclear cells and tonsillar B-cell enriched fractions. In contrast, IFN-α and IFN-β did not enhance or suppress SAC-stimulated blastogenesis in either lymphocyte preparation. The enhancing effects specific to IFN-γ were more remarkable in cultures stimulated with a suboptimal dose (0·002%) of SAC than when the optimal dose (0·005%) was employed.

INTRODUCTION

In addition to its well-established anti-viral effects, interferon (IFN) has been demonstrated to affect a variety of immune responses, including the proliferative response of lymphocytes to polyclonal mitogens and antigens (Blomgren, Strander & Cantell, 1974; Miörner *et al.*, 1978; Einhorn *et al.*, 1979, 1983; Aoki *et al.*, 1984). There are three major types of IFN, termed IFN-α (leucocyte), IFN-β (fibroblast) and IFN-γ (immune), that can be distinguished antigenically and physicochemically. Of these, IFN-γ could be naturally anticipated to play the most important role in the regulatory biology of immunocompetent cells in view of its characteristic as a lymphokine typically produced in a wide range of T-cell responses (Balkwill, 1985). With the recent availability of highly purified preparations of natural and recombinant human IFNs for clinical trials, they can be used for comparative studies of their influence on immune responses. In a series of investigations to examine whether three highly purified IFN preparations might exert differential effects on *in-vitro* immune responses, we have previously shown that human IFN-γ, distinct from IFN-α and IFN-β, enhances the pokeweed mitogen (PWM)-induced blastogenic response of human peripheral lymphocytes, suggesting that IFN-γ might specifically exert some effects on the B-cell lineage (Aoki, Ohno & Yamamoto, 1985). These observations prompted us to investigate further whether the B-cell response to *Staphylococcus aureus* Cowan strain 1 (SAC), a well-defined T-cell independent B-cell activator, could be specifically enhanced by IFN-γ. In the present study, the three natural human IFNs were compared with regard to their effects on the SAC-induced proliferative response of human peripheral lymphocytes as well as of B-cell enriched populations obtained from human tonsils.

Correspondence: Dr N. Aoki, Dept. of Medicine, Kinki University School of Medicine, Sayama, Minamikawachi, Osaka 589, Japan.

MATERIALS AND METHODS

Peripheral mononuclear cells

Peripheral mononuclear cells (PMC) were obtained from heparinized blood of healthy donors using Ficoll–Hypaque (Pharmacia, Piscataway, NJ) separation as reported previously (Aoki & DeGroot, 1979), washed three times in 0·15 M phosphate-buffered saline (PBS) and resuspended in RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (FCS, Gibco) at 2×10^6 cells/ml. The cells were counted and tested for viability by the trypan blue dye exclusion test. The cell populations thus obtained were composed of 90–93% lymphoid cells and 7–10% monocytes.

Tonsillar mononuclear cells

Tonsillar mononuclear cells (TMC) were tested from fresh sections of human tonsils obtained at tonsillectomy from patients with chronic tonsillitis and passed through a 105-μm stainless mesh. Red blood cells present in the preparation were lysed in 0·83% NH_4Cl in 17 mM Tris for 5 min at room temperature. Intact cells were then washed three times in PBS and suspended in RPMI-1640 medium containing 10% FCS (10% FCS RPMI-1640) at $0{\cdot}5–1 \times 10^7$ cells/ml. Five ml of the suspension were incubated in FCS-coated plastic petri-dishes

(Nunc no. 150350, Roskilde, Denmark) at 37° for 2 hr in a humidified 5% CO_2 atmosphere to remove adherent cells (Kumagai *et al.*, 1979). Subsequently, non-adherent cells were collected from the dishes, washed three times in PBS and resuspended in 10% FCS RPMI-1640 at 0·5–1 × 10^7 cells/ml.

Tonsillar B-cell enriched fractions

In order to deplete T cells, TMC were allowed to rosette with 2-aminoethyl isothiouroniumbromide-treated sheep red blood cells (SRBC), essentially as described in the literature (Saxon, Feldhaus & Robins, 1976). In brief, 5–20 ml of TMC suspension were mixed with an equal volume of 2% SRBC in PBS in a 15-ml of 50-ml plastic tube (Falcon, Cockeysville, MD) and incubated at 4° for 2 hr after centrifugation at 60 ***g*** for 5 min. Then, pelletted cells were gently resuspended in 3–6 ml of PBS by rotating the tube manually, and layered over a Ficoll–Hypaque gradient which was then centrifuged at 400 ***g*** for 30 min. With these procedures T cells formed rosettes, which went to the bottom of the tube, while B cells remained in the interface. The cells in the interphase were pipetted out with a Pasteur pipette, washed three times in PBS, and resuspended in 10% FCS RPMI-1640. Following three cycles of the rosetting and centrifugation procedure to remove T cells, the cells recovered in 10% FCS RPMI-1640 were again incubated in FCS-coated plastic dishes to remove adherent cells as described above. Non-adherent cells were washed twice in PBS and finally suspended in 10% FCS RPMI-1640 to be designated the tonsillar B-cell enriched fractions. The final preparations were checked for the presence of residual T cells and adherent cells by staining with FITC-labelled OKT3 monoclonal antibody (Ortho Pharmaceutical, Raritan, NJ) and Leu-M3 monoclonal antibody (Becton-Dickinson, Mountain View, CA), respectively, as well as by measuring the blastogenic response of the preparations to T-cell mitogens.

Interferon preparations

Highly purified IFN preparations for clinical trials were kindly supplied by Dr H. Morise, Green Cross Corporation, Osaka, Japan). IFN-α was produced in Sendai virus-infected Namalva (human lymphoblastoid) cells (Klein *et al.*, 1984); IFN-β was induced in human foreskin fibroblasts by poly I:C (Raj & Pitha, 1981); and IFN-γ was prepared by stimulation of human leucocytes with phytohaemagglutinin (de Ley *et al.*, 1980) and successive purification with affinity chromatography using monoclonal anti-IFN-γ antibody. The specific activity was 1·8 × 10^7 IU/mg protein for IFN-α, 3·3 × 10^7 IU/mg protein for IFN-β, and 2·9 × 10^7 IU/mg protein for IFN-γ, respectively.

Cell culture conditions

The cells obtained from the peripheral blood or tonsils were cultured in 10% FCS RPMI-1640 at 37° in 5% CO_2 in moist air. The incubation was carried out in flat-bottomed microculture plates (Falcon), each well contained 10^5 cells suspended in 0·25 ml of the culture medium with or without additives. The additives included phytohaemagglutinin P (PHA, Gibco), pokeweed mitogen (PWM, Gibco), protein A (PA, Pharmacia), *Staphylococcus aureus* Cowan strain 1 (SAC, Calbiochem, La Jolla, CA) and IFNs. They were dissolved in the medium and adjusted to a 1:100 dilution for both PHA and PWM, 10 μg/ml for PA, 0·002% and 0·005% for SAC, and varying doses ranging from 10 to 1000 IU/ml for IFNs, in a final volume of 0·25 ml of the cell suspension. IFN-α, IFN-β and IFN-γ were compared with respect to their effects on SAC-induced blastogenesis with equal units IU) of anti-viral activity ranging from 10 to 1000 IU/ml. Incubation was performed as above for 3–days. The cells in each well were pulsed with ^{125}I-iododeoxsyuridine (^{125}I-IUdR, New England Nuclear, Boston, MA) at a concentration of 0·4 μCi/ml for the last 4 hr of culture to monitor DNA synthesis due to the lymphoproliferative response. The cells were then collected on glass wool filters with the use of an automated cell harvester (Skatron, Lier, Norway). The radioactivity of ^{125}I-IUdR incorporated into DNA was measured in a gamma counter and expressed as counts per minute (c.p.m.).

The mean c.p.m. obtained from four to six replicate cultures was calculated. Statistical evaluation for differences between the two groups was performed using the Student's *t*-test.

RESULTS

The effects of natural human IFN-α, IFN-β and IFN-γ on the SAC-stimulated lymphoproliferative response of peripheral mononuclear cells are shown in Fig. 1. The blastogenic response of PMC to the optimal dose of SAC (0·005%) was increased more than 10-fold as compared with the blastogenesis in the control which contained no SAC. While the addition of IFN-α or IFN-β to SAC-stimulated cultures did not cause significant enhancement of the reaction at any IFN dose of 10, 100 and 1000 IU/ml, IFN-γ markedly enhanced the SAC response in a dose-dependent manner in the same dose range. It is worthy of note that IFN-α and IFN-β did not suppress the SAC blastogenesis at all in the present study, contracting to their marked suppression of PHA- or PWM-stimulated blastogenesis as

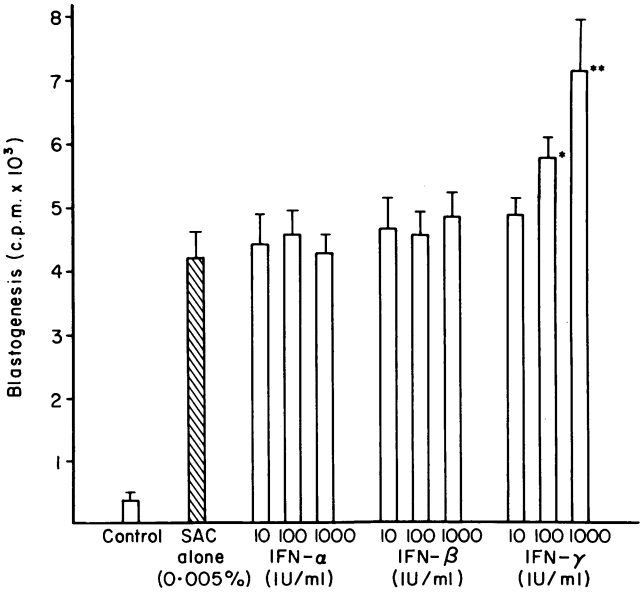

Figure 1. Effect of different species of IFNs on the SAC-induced blastogenic response of PMC. PMC (10^5) were cultured in 0·25 ml medium for 3–4 days with 0·005% SAC in the absence or presence of IFNs at indicated concentrations. The cells were pulsed with ^{125}I-IUdR for the last 4 hr of culture. Values represent the mean ± SD of five to six replicate cultures. $^*P < 0·05$, $^{**}P < 0·01$, as compared with cultures stimulated singly with SAC (the shaded column).

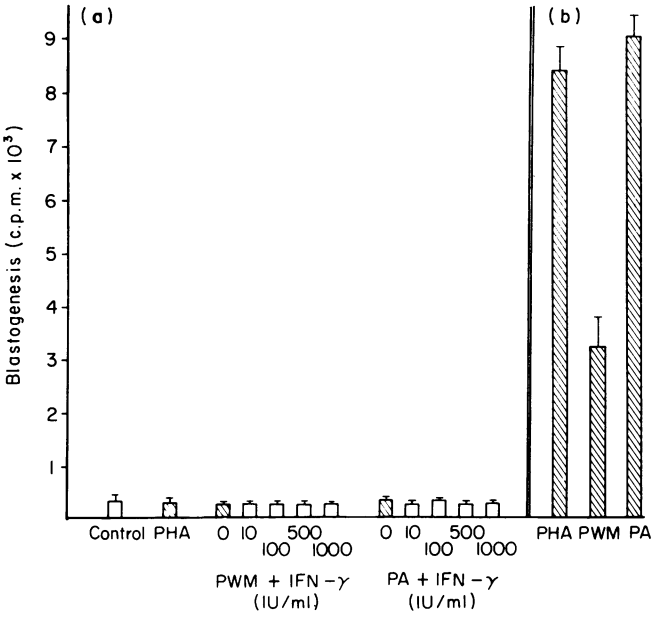

Figure 2. Responsiveness of fractionated tonsillar B cells (a) and unfractionated tonsillar mononuclear cells (b) to PHA, PWM and PA. Separation and fractionation of tonsillar mononuclear cells were performed as described in the Materials and Methods. Each culture including 10^5 cells in 0·25 ml of culture medium was stimulated with PHA (1:100), PWM (1:100) or PA (10 μg/ml) for 3–4 days. IFN-γ was added to PWM-stimulated cultures and PA-stimulated cultures by the doses indicated. DNA synthesis was assessed as described in Fig. 1. Values represent the mean ± SD of six replicate cultures. Shaded columns show the response of each mitogen in the absence of IFN-γ. The results with unfractionated tonsillar cells are shown for comparison (b).

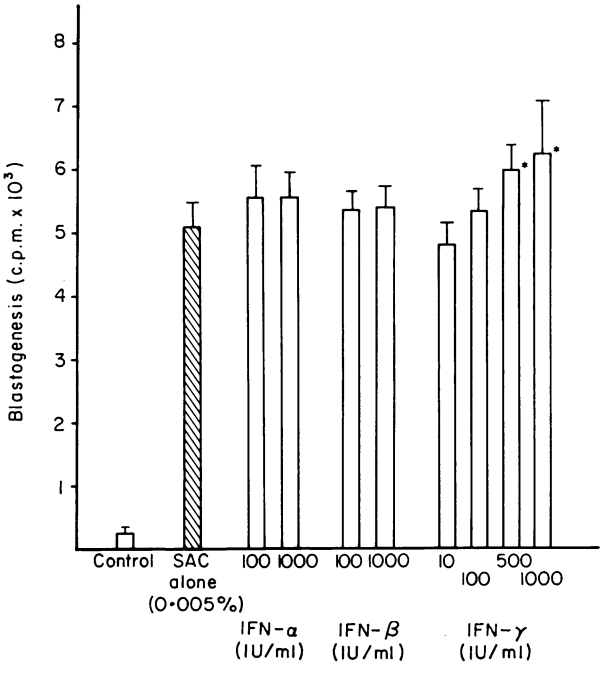

Figure 3. Efffect of different species of IFNs on SAC-induced blastogenic response of tonsillar B-cell enriched fractions. Fractionated tonsillar B cells (10^5) were stimulated with 0·005% SAC in the absence or presence of IFNs. IFNs were added to SAC-stimulated cultures by the doses indicated. The assays were performed as described in Fig. 2. Values represent the mean ± SD of five to six replicate cultures. $^*P < 0·05$, as compared with cultures stimulated singly with SAC (the shaded column).

reported previously (Aoki *et al.*, 1985). In addition, none of the IFNs, when added singly to PMC cultures, significantly altered the basal blastogenesis of the cultures. For instance, PMC culture incubated singly with 1000 IU of IFN per ml gave a blastogenic response of 342 ± 99 c.p.m. (mean ± SD) for IFN-α, 357 ± 102 c.p.m. for IFN-β and 325 ± 110 c.p.m. for IFN-γ, while the response in PMC cultures without IFN was 335 ± 96 c.p.m. In order to explore these findings further, comparative studies among the three IFNs as to their effects on the SAC-induced response were performed using tonsillar-enriched B cells instead of PMC. At the beginning, the characteristics of the B-cell preparations employed in the present experiments were examined. Tonsillar B-cell enriched populations were found to include less than 0·4% OKT3-positive cells and less than 0·7% Leu-M3-positive cells in repeat experiments (data not shown). The responsiveness of the B-cell preparations to various stimulators is shown in Fig. 2. It is clear that the preparations did not show blastogenic response to PHA, PWM or PA, no matter whether the mitogens were employed alone or in combination with IFN-γ (Fig. 2a). Additionally, it was confirmed that the B-cell populations did not respond to IFN-γ *per se* at concentrations of 10, 100, 500 and 1000 IU/ml (data not shown). In contrast, crude or unfractionated TMC responded markedly to PHA, PWM and PA, showing a high response to PA and PHA and a moderate response to PWM (Fig. 2b). Using thus purified B cells, IFN effects on the SAC-stimulated lymphocyte blastogenesis were examined (Fig. 3). As seen with PMC, SAC blastogenesis in enriched B cells was also enhanced in a dose-dependent manner in the presence of IFN-γ, but was not affected at all by the addition of IFN-α and IFN-β. The SAC-induced response was significantly enhanced in the presence of IFN-γ at concentrations of 500 and 1000 IU/ml. In another experiment using enriched B cells obtained from a different patient, the effect of IFN-γ on SAC-induced blastogenesis was investigated at two concentrations of SAC, suboptimal (0·002%) and optimal (0·005%), as shown in Fig. 4. It is clear that the B-cell preparations did not respond to PHA, PWM or PA in agreement with the data in Fig. 2, while they responded substantially and in a dose-related manner to SAC, accompanied by marked enhancement of the response by IFN-γ. This IFN-γ-induced enhancement of SAC-stimulated blastogenesis was demonstrated at both concentrations of SAC examined, and was highly significant at each IFN dose ranging from 50 to 1000 IU/ml as compared with the response in SAC-stimulated cultures without IFN-γ. In particular, the enhancing effect of IFN-γ was greater in culture conditions in which the SAC concentration was suboptimal than when it was optimal.

DISCUSSION

We have extended our previous study showing that natural human IFN-γ, but not natural human IFN-α and IFN-β, unexpectedly enhanced the lymphoproliferative response of PMC to PWM. PWM is a T-cell dependent B-cell stimulator, and this suggested that IFN-γ might possess activity specifically

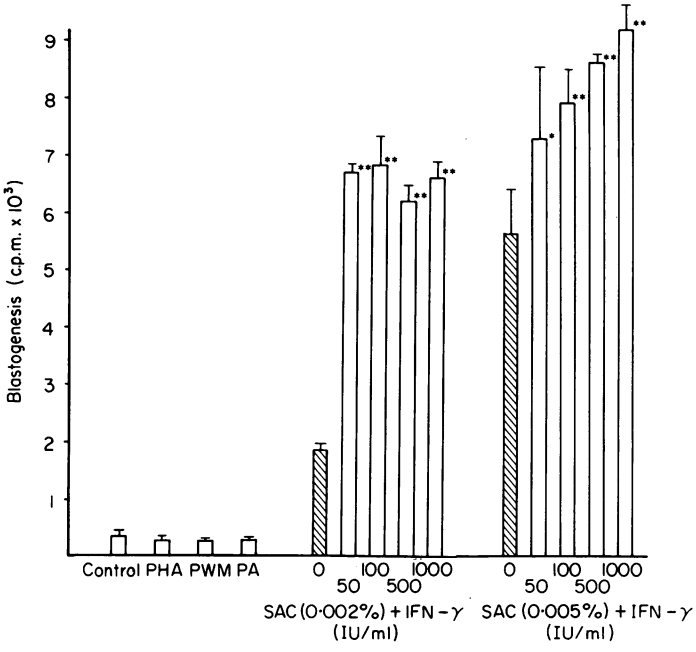

Figure 4. Effect of IFN-γ on SAC-induced blastogenic response of tonsillar B-cell enriched fractions. The responses of the B-cell populations to PHA (1 : 100), PWM (1 : 100) and PA (10 μg/ml) are shown on the left side of the panel. The responses of the same B-cell preparations to 0·002% SAC and 0·005% SAC are shown in the middle and the right of the panel, respectively. IFN-γ was added to these SAC cultures by the doses indicated. The assays were performed as described in Fig. 2. Values represent the means $\pm$ SD of four to six replicate cultures. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, as compared with cultures stimulated singly with SAC as shown by a shaded column in each group.

affecting the B-cell lineage (Aoki *et al.*, 1985). The present study, more definitely designed to evaluate B-cell responses, has clearly shown that natural human IFN-γ can exclusively enhance B-cell blastogenesis. Unlike PWM, which is a T-cell dependent B-cell mitogen, SAC is well established as a B-cell specific mitogen (Falkoff, Zhu & Robins, 1982). In PMC cultures stimulated with SAC, blastogenic response was found to be significantly increased in the presence of IFN-γ but not with IFN-α or IFN-β. The difference in IFNs as such is broadly in concordance with the previous findings with PWM, although in PWM-induced blastogenesis there was suppression of the response in the presence of IFN-α and IFN-β. While it is generally accepted that IFN can inhibit proliferation of normal and neoplastic cells (Taylor-Papadimitriou & Rozengurt, 1985), this does not seem to be true for B-cell proliferation as shown in the present study.

The IFN-γ-specific enhancement of SAC-induced blastogenesis was conclusively demonstrated in tonsillar B-cell enriched populations that were deleted of T cells and adherent cells. The enhancement due to IFN-γ was more marked at a suboptimal concentration of SAC than at its optimal dose. The enriched B-cell populations employed in the present study were highly purified as shown by the study with monoclonal antibodies and by their inability to respond to the potent T-cell mitogens such as PHA and PA (Schuurman, Gelfand & Dosch, 1980) as well as to PWM. Therefore, the observed effects due to IFN-γ would not involve the production of any T-cell derived factors but rather seem to be directly concerned in the B-cell activation phase. Since similar enhancing effects were observed even when recombinant human IFN-γ (kindly provided by Shionogi Pharmaceutical, Osaka, Japan) was used instead of natural human IFN-γ, the enhancing activity seems to be intrinsic to the IFN-γ molecule (unpublished observations). These results, taken together with our previous findings (Aoki *et al.*, 1985), suggest that lymphocyte blastogenesis involving proliferation of any B cell could be enhanced in the presence of IFN-γ, probably owing to its direct effects on B-cell activation.

Little is known at present about the exact mechanism of the humoral and acceptor events involved in the SAC-stimulated B-cell response, although various soluble factors have been reported recently to affect B-cell activation and subsequent differentiation into immunoglobulin-secreting cells. They include interleukin-1 (IL-1), interleukin-2 (IL-2), B-cell growth factors (BCGFs), T-cell replacing factors (TRFs), IFN-γ, and possibly other B-cell stimulating factors (Hoffmann, 1980; Emmrich, Moll & Simon, 1985; Lantz *et al.*, 1985; Howard, 1985; Swain & Dutton, 1985; Liebson *et al.*, 1984; Sidman *et al.*, 1984a; Sidman, Paige & Schreier, 1984b).

The information that IFN-γ can enhance or induce the proliferation of B cells stimulated by SAC could be interpreted in several ways. Firstly, IFN-γ might have BCGF activity. This would be in analogy to IL-2 which has recently been demonstrated to exert direct effects on B cells in a way similar to BCGF (Emmrich *et al.*, 1984; Lantz *et al.*, 1985; Mond *et al.*, 1985), presumably mediated through the IL-2 receptor demonstrated on B cells (Tsudo, Uchiyama & Uchino, 1984). In the present study, IFN-γ had little effect on resting B cells but dramatically enhanced the proliferation of B cells activated by SAC, as shown in Figs 3 and 4, indicating that IFN-γ might also be a BCGF to enhance the proliferation of activated B cells through the IFN-γ receptor expressed on the cells. As these IFN-γ effects seem to be related to the early phase of B-cell activation, they should be differentiated from another IFN-γ effect, in which it has a B-cell maturing activity or is a major component of TRF activities that would be exerted in the late phase of the antibody-producing

response (Liebson *et al.*, 1984; Sidman *et al.*, 1984a). Secondly, IL-1 might be involved in the observed enhancement of the B-cell response, considering that IL-1 can be produced by B cells as recently reported (Matsushima *et al.*, 1985) as well as by contaminant, if very few, accessory cells. IFN-γ could augment IL-1 effects by enhancing IL-1 release (Newton, 1985). It is also possible that IFN-γ might intensify the expression of putative IL-1 receptors on B cells. Thirdly, other as yet unidentified molecules distinct from IL-1 might be induced by IFN-γ and thereby contribute to the enhancement of the B-cell response by functioning as accessory or B-cell stimulating factors. Finally, it cannot be entirely excluded that there was an enhancement of IL-2 effects, even in the present B-cell cultures, considering that there are IL-2 receptors on B cells (Tsudo *et al.*, 1984) and that IFN-γ can induce the IL-2 responsiveness of T cells (Palladino, 1983), monocytes (Herrmann *et al.*, 1985) and leukaemic cells (Herrmann *et al.*, 1985), although the data are not available as yet for B cells, and it appears to be contradictory to speculate IL-2 prduction in the present system. Experiments to test these possibilities are currently underway.

ACKNOWLEDGMENTS

The authors are grateful to Dr H. Morise and Dr H. Arimura of the Green Cross Corporation, Osaka, for providing the interferon preparations, and to Ms T. Aoki for her secretarial assistance.

REFERENCES

Aoki N. & DeGroot L.J. (1979) PPD-induced blastogenesis is autoregulated by suppressor cells generated *in vitro*. *Experientia*, **35,** 1515.

Aoki N., Maruyama Y., Ohno Y. & Azuma Y. (1984) Indomethacin augments inhibitory effects of interferons on lymphoproliferative response. *Immunol. Lett.* **7,** 321.

Aoki N., Ohno Y. & Yamamoto T. (1985) Gamma interferon enhances mitogenic responses induced by pokeweed mitogen. *Immunol. Lett.* **10,** 87.

Balkwill F.R. (1985) In: *Interferons. Their Impact in Biology and Medicine* (ed. J. Taylor-Papadimitriou), p. 61. Oxford University Press, New York.

Blomgren H., Strander H. & Cantell K. (1974) Effect of human leukocyte interferon on the response of lymphocytes to mitogenic stimuli *in vitro*. *Scand. J. Immunol.* **3,** 697.

Einhorn S., Blomgren H., Cantell K. & Strander H. (1979) Effect of prolonged *in vivo* administration of leukocyte interferon on the mitogen responsiveness of human lymphocytes. *Acta Med. Scand.* **206,** 345.

Einhorn S., Blomgren H., Einhorn N. & Strander H. (1983) *In vitro* and *in vivo* effects of interferon on the response of human lymphocytes to mitogens. *Clin. exp. Immunol.* **51,** 369.

Emmrich F., Moll H. & Simon M.M. (1985) Recombinant human interleukin 2 acts as a B cell growth and differentiation promoting factor. *Immunobiology*, **169,** 97.

Falkoff R.J.M., Zhu L. & Robins R.A. (1982) Separate signals for human B cell proliferation and differentiation in response to *Staphylococcus aureus*: evidence for a two-signal model of B cell activation. *J. Immunol.* **129,** 97.

Herrmann F., Cannistra S.A., Levine H. & Griffin J.D. (1985) Expression of interleukin 2 receptors and binding of interleukin 2 by gamma interferon-induced human leukemic and normal monocytic cells. *J. exp. Med.* **162,** 1111.

Hoffmann M.K. (1980) Macrophages and T cells control distinct phases of B cell differentiation in the humoral immune response *in vitro*. *J. Immunol.* **125,** 2076.

Howard M. (1985) In: *Contemporary Topics in Molecular Immunology* (eds S. Gillis and F.P. Inman), Vol. 10, The Interleukins, p. 181. Plenum Press, New York.

Klein F., Ricketts R.T., Jones W.I. & Clark P. (1984) Induction potential of Sendai virus and Newcastle disease virus for human lymphoblastoid interferon production. *J. Interferon Res.* **4,** 243.

Kumagai K., Itoh K., Hinuma S. & Tada M. (1979) Pretreatment of plastic petri dishes with fetal calf serum. A simple method for macrophage isolation. *J. immunol. Meth.* **29,** 17.

Lantz O., Grillot-Courvalin C., Schmitt C., Fermand J.-P. & Brouet J.-C. (1985) Interleukin 2-induced proliferation of leukemic human B cells. *J. exp. Med.* **161,** 1225.

deLey M., van Damme J., Claeys H., Weening H., Heine J.W., Billau A., Vermylen C. & de Somer P. (1980) Interferon induced in human leukocytes by mitogens: production, partial purification and characterization. *Eur. J. Immunol.* **10,** 877.

Liebson H.J., Gefter M., Zlotnik A., Marrack P. & Kappler J.W. (1984) Role of γ-interferon in antibody-producing responses. *Nature (Lond.)*, **309,** 799.

Matsushima K., Procopio A., Abe H., Scala G., Ortaldo J.R. & Oppenheim J.J. (1985) Production of interleukin 1 activity by normal human peripheral blood B lymphocytes. *J. Immunol.* **135,** 1132.

Miörner H., Landstrom E., Larner E., Larsson I., Lundegren E. & Strannegard O. (1978) Regulation of mitogen-induced lymphocyte DNA synthesis by human interferon of different origins. *Cell. Immunol.* **35,** 15.

Mond J.J., Thompson C., Finkelman F.D., Farrar J., Schaefer M. & Robb R.J. (1985) Affinity-purified interleukin 2 induces proliferation of large but not small B cells. *Proc. natl. Acad. Sci. U.S.A.* **82,** 1518.

Newton R.C. (1985) Effect of interferon on the induction of human monocyte secretion of interleukin-1 activity. *Immunology*, **56,** 441.

Palladino M.A., Svedersky L.P., Shepard H.M., Pearlstein K.T., Vilcek J. & Scheid M.T. (1983) In: *Interferon: Research, Clinical Application and Regulatory Consideration* (eds K. C. Zoon, P.D. Noguchi and T.-Y. Liu), p. 139. Elsevier, New York.

Raj N.B.K. & Pitha P.M. (1981) Analysis of interferon mRNA in human fibroblast cells induced to produce interferon. *Proc. natl. Acad. Sci. U.S.A.* **78,** 7426.

Saxon A., Feldhaus J. & Robins R.A. (1976) Single step separation of human T and B cells using AET treated SRBC rosettes. *J. immunol. Meth.* **29,** 17.

Schuurman R.K.B., Gelfand E.W. & Dosch H.-M. (1980) Polyclonal activation of human lymphocytes *in vitro*. 1. Characterization of the lymphocyte response to a T cell-independent B cell mitogen. *J. Immunol.* **125,** 820.

Sidman C.L., Marshall J.D., Shultz L.D., Gray P.W. & Johnson H.M. (1984a) γ-interferon is one of several direct B cell-maturing lymphokines. *Nature (Lond.)*, **309,** 801.

Sidman C.L., Paige C.J. & Schreier M.H. (1984b) B cell maturation factor (BMF): a lymphokine or family of lymphokines promoting the maturation of B lymphocytes. *J. Immunol.* **132,** 209.

Swain S.L. & Dutton R.W. (1985) In: *Contemporary Topics in Molecular Immunology* (eds S. Gillis and F. P. Inman) Vol. 10, The Interleukins, p. 219. Plenum Press, New York.

Taylor-Papadimitrious J. & Rozengurt E. (1985) In: *Interferons. Their Impact in Biology and Medicine* (ed. J. Taylor-Papadimitriou), p. 81. Oxford University Press, New York.

Tsudo M., Uchiyama T. & Uchino H. (1984) Expression of TAC antigen on activated normal human B cells. *J. exp. Med.* **160,** 612.