Immunology 1990 **70** 100–105

Epstein–Barr virus-immortalized B cells produce IL-6 as an autocrine growth factor

T. YOKOI, T. MIYAWAKI, A. YACHIE, K. KATO, Y. KASAHARA & N. TANIGUCHI *Department of Pediatrics, School of Medicine, Kanazawa University, Kanazawa, Japan*

Accepted for publication 12 January 1990

SUMMARY

The continuous proliferation of Epstein–Barr virus (EBV)-immortalized B cells is enhanced by autocrine as well as paracrine growth factors. In the present study, the possibility that EBV-immortalized B cells might produce interleukin-6 (IL-6) proteins in an autocrine manner was examined. It was found that culture supernatants from EBV-transformed B cells, but not from Burkitt's lymphoma lines, augmented the proliferation of an IL-6-dependent murine hybridoma clone, MH60.BSF2. This growth-promoting activity for hybridoma cells found in culture supernatants of EBV-transformed B cells was specifically neutralized by rabbit anti-recombinant (r) IL-6 antibody. The IL-6 activity in culture supernatants of EBV-transformed B cells, though much less than that of lipopolysaccharide (LPS)-stimulated monocytes, was increased by the addition of phorbol myristate acetate. Western blot experiments using rabbit anti-rIL-6 antiserum demonstrated that supernatants from cultured EBV-transformed B cells contained the distinct forms of IL-6, with a peak of 23,000 MW. When examined by *in situ* hybridization analysis, it was found that IL-6 mRNA were expressed on EBV-transformed B cells. It was noted that a fraction, but not all, of these cells expressed IL-6 mRNA strongly, implying their cell cycle-dependent expression. In addition, it was shown that rIL-6 promoted the growth of EBV-transformed B cells at low cell densities. The results suggest that IL-6 serves as an autocrine growth factor in EBV-transformed B cells.

INTRODUCTION

Epstein–Barr virus (EBV) is a ubiquitous human herpes virus etiologically associated with both benign (acute infectious mononucleosis) and malignant (Burkitt's lymphoma and nasopharyngeal carcinoma) disorders (Thorley-Lawson, 1988), but the exact role of EBV infection in malignancy remains to be understood. It has been demonstrated that EBV can transform and immortalize a broad spectrum of B-lineage cells, including mature B cells, pre-B cells, and putative Ig^- B precursors, into continuously growing lymphoblastoid cell lines (Tosato & Blaese, 1985; Kubagawa *et al.*, 1988). It is widely accepted that autocrine secretion of growth factors may occur in certain kinds of tumour cell (Sporn & Roberts, 1985). Similarly, there is evidence that the autonomous growth of EBV-transformed B cells is dependent, at least in part, upon the presence of autocrine growth factors (Blazar, Sutton & Strome, 1983; Gordon *et al.*, 1984), although the mechanisms of B-cell transformation and immortalization by EBV are largely unknown. Previous studies have identified a factor similar to T cell-derived B-cell growth factor (Gordon *et al.*, 1984), interleukin (IL)-1-like factors (Wakasugi *et al.*, 1987), or a soluble form of CD23 (Swendeman & Thorley-Lawson, 1987) as candidates for these autocrine growth factors. Recently, it has been shown that EBV-transformed B-cell lines express receptors for IL-6 (Taga *et al.*, 1987) and that IL-6 is able to promote their proliferation and growth (Tosato *et al.*, 1988a, b). However, the capability of EBV-transformed B cells to produce IL-6 has not yet been documented.

In this report, it is shown that the EBV-transformed B-cell lines express IL-6 at both the mRNA and protein levels. The *in situ* hybridization technique demonstrates that a proportion of EBV-transformed B cells express the IL-6 mRNA. The proliferation of EBV-transformed B cell was enhanced by addition of IL-6 at low cell densities. The results indicate the possibility that IL-6 acts as one of the autocrine growth factors for EBV-immortalized B cells.

Abbreviations: EBV, Epstein–Barr virus; FCS, fetal calf serum; IL, interleukin; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PMA, phorbol myristate acetate; r, recombinant.

Correspondence: Dr T. Miyawaki, Dept. of Pediatrics, School of Medicine, Kanazawa University, 13-1 Takara-Machi, Kanazawa, Ishikawa, 920, Japan.

MATERIALS AND METHODS

Cells

Burkitt's lines, Raji and Daudi, and an established EBV-transformed cell line, RPMI-1788, and other B-cell lines, IM-9

and BALL, were obtained from the Japanese Cancer Research Resources Bank, Tokyo. TK is an EBV-negative B-lymphoblastoid cell line, obtained from a lymphoma patient at this laboratory. Several EBV-transformed B-cell lines, such as TM-1, HS-2 and others, were established by infecting peripheral blood B cells from healthy adult donors with the B95-8 strain of EBV, as described previously (Miyawaki *et al.*, 1988). All cell lines were maintained in culture medium consisting of RPMI-1640 (Gibco, Grand Island, NY) with 10% fetal calf serum (FCS; Flow Laboratories, North Ryde, Australia), 2 mM L-glutamine, 10 mM HEPES (Gibco), 50 μM 2-mercaptoethanol, 200 U/ml penicillin and 10 μg/ml gentamicin. To collect culture supernatants for IL-6 assay, cells were cultured at 5×10^5 cells/ml in 24-well tissue culture plates (2 ml/well; Falcon, Lincoln Park, NJ) with or without 20 ng/ml phorbol myristate acetate (PMA; Sigma Chemical Co., St Louis, MO), at 37° for 48 hr in a humidified environment of 5% CO_2 in air.

IL-6 assay

IL-6 activity in the culture supernatants was measured by a sensitive colorimetric assay using an IL-6-dependent murine hybridoma clone, MH60.BSF2 (kindly provided by Drs T. Hirano and T. Kishimoto, Osaka University, Osaka) as described elsewhere (Matsuda, Hirano & Kishimoto, 1988; Ueno *et al.*, 1989). The hybridoma clone was maintained in medium supplemented with 60 μg/ml tylocine (Gibco) (culture medium) in the presence of human recombinant (r)IL-6 (provided by Drs T. Hirano and T. Kishimoto). The cells were harvested, washed three times in culture medium and further preincubated in the absence of IL-6 for 6 hr at 37°. Subsequently, cells (10^4) in 100 μl of culture medium were cultured together with serial dilutions of samples in a final volume of 200 μl in 96-well flat-bottomed microtitre plates (Corning Glass Works, Corning, NY). The cultures were carried out at 37° in 5% CO_2/air. After 44 hr of culture, 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co.) was added to each well to a concentration of 0·5 mg/ml. After further incubation at 37° for 4 hr, the unreacted dye and medium were discarded, following centrifugation at 200 *g* for 5 min. Then 100 μl of acidified isopropylalcohol (0·04 N HCl in isopropylalcohol) were added to each well. After leaving the plates for 20 min, the plates were briefly shaken and 20 μl of 3% SDS in H_2O were added to each well. The optical density of MTT formazan was measured at 550 nm by an automatic microplate spectrophotometer. In all assays, a titration curve of rIL-6 as the standard was included. Recombinant preparations of human IL-6 used in this study had a specific activity of $5{\cdot}2 \times 10^6$ U/mg of protein determined by the augmentation of IgM secretion by SKW-C14 cells (Hirano *et al.*, 1986).

Neutralization with anti-IL-6 antiserum

To confirm the specificity of IL-6 activity, neutralization experiments were carried out with the rabbit anti-rIL-6 IgG (provided by Drs T. Hirano and T. Kishimoto). The culture supernatants were mixed with various concentrations of rabbit anti-rIL-6 IgG or non-immune rabbit IgG, and incubated for 1 hr at 37°. Then the neutralized samples were tested for the ability to induce the proliferation of MH60.BSF2 cells described above.

Western blot analysis

For Western blot analysis, the cells were cultured at 5×10^5 cells/ml in 10 ml of serum-free culture medium in 50 ml tissue culture flasks (Costar, Cambridge, MA). IL-6 activity produced by EBV-transformed cells in this culture condition was confirmed to be similar to those seen in FCS-containing medium. Culture supernatants were concentrated to a fiftieth of the original volume, with a 10,000 molecular cut off, by ultrafiltration (Centricut; Kurabo, Osaka). These concentrated supernatant materials were lyophilized, resuspended in 1 × Laemmli buffer, and electrophoresed through an SDS–polyacrylamide gradient (10–20%) gel, and transferred to a nitrocellulose paper using a semi-dry electrophoretic apparatus (Horizeblot, Atto Corporation, Tokyo). An immunoperoxidase procedure with rabbit anti-rIL-6 antiserum (provided by Dr L. T. May, Rockefeller University, New York, NY) was used to detect immunospecific proteins, as described elsewhere (May *et al.*, 1988), except that a 1:1000 dilution of peroxidase conjugated affinity-purified goat anti-rabbit IgG antibody (Tago Inc., Burlingame, CA) was used. For the colour reaction, the blots were soaked in a solution of 1 mg diaminobenzidine per ml of 0·01% H_2O_2 in 50 mM Tris-HCl, pH 7·6 . The reaction was terminated after 10–20 min by washing with tap water.

Preparation of RNA probe

To prepare the RNA probes for IL-6 mRNA, the TaqI-XbaI DNA fragment of pBSF.38.1 (IL-6 cDNA; provided by Drs T. Hirano and T. Kishimoto) (Hirano *et al.*, 1986) was inserted into the AccI-XbaI site of the transcription vector pGEM3Z (Promega Biotec, Madison, WI). The recombinant plasmid was linearized with the appropriate restriction enzyme. Radioactive sense and anti-sense RNA probes were generated using SP6 and T7 RNA polymerase and ^{35}S-UTP (Amersham Japan, Tokyo). A specific activity of 2×10^8 c.p.m./μg DNA template was obtained.

In situ *hybridization*

In situ hybridization with ^{35}S-labelled RNA probe was carried out as described before (Kato *et al.*, 1990). Briefly, the cells were cytospun onto the pretreated glass slides, air-dried, fixed in freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7·4), acetylated at pH 8·0 in 0·1 M triethanolamine and 0·25% acetic anhydride, rinsed in 0·1 M Tris-HCl (pH 7·0) and 0·1 M glycine, and then dehydrated in graded ethanol solutions.

Ten microliters of quenched hybridization mixture (10^6 c.p.m. of ^{35}S-labelled RNA probe, 50% formamide, 2 × SSC, 10 mM dithiothreitol, sheared salmon sperm DNA, yeast tRNA and BSA) were loaded on each slide. The hybridization was carried out at 50° overnight, and washed in 50% formamide/2 × SSC at 54° for 1 hr, followed by several rinses in 2 × SSC. After the ribonuclease treatment, slides were again rinsed in 50% formamide/2 × SSC at 54° and dehydrated in ethanol. For autoradiography, the slides were coated with NR-M2 emulsion (Konica Corporation, Tokyo), diluted 1:1 with distilled water, exposed for 10 days at 4°, then developed in Kodak D19 for 5 min, rinsed in water, and fixed for 5 min in Kodak fixer. The slides were washed with tap water, and stained with Giemsa's stain. The cells were considered positive for gene expression when they exhibited more grains than cells with the highest

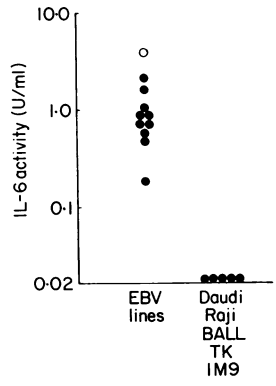

Figure 1. IL-6 activity in culture supernatants from various cell lines. EBV-transformed B cells and other B-cell lines were cultured at 5×10^5 cells/ml for 48 hr, and IL-6 activity in culture supernatants was evaluated by a colorimetric assay using an IL-6-dependent murine hybridoma clone, MH60.BSF2, as described in the Materials and Methods. (○) indicates the EBV-transformed cell line, RPMI-1788.

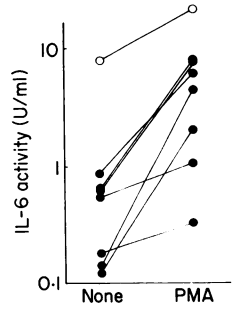

Figure 2. PMA augments IL-6 production by EBV-transformed B cells. Several EBV-transformed B-cell lines were cultured with or without 20 ng/ml of PMA. An open circle and closed circles indicate RPMI-1788 and other EBV-transformed B-cell lines, respectively.

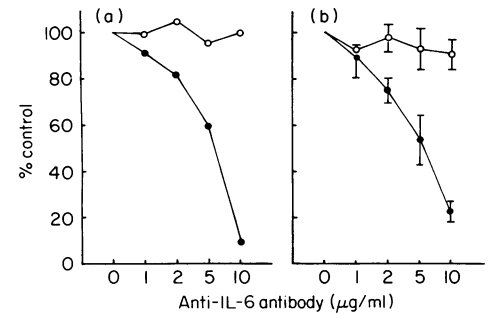

Figure 3. Neutralization of IL-6 activity in culture supernatants of EBV-transformed B cells by anti-rIL-6 antibody. Appropriate amounts of rIL-6 (0·2 U/ml) (a) or culture supernatants of EBV- transformed B cells (20%, v/v) (b), which induced about 70% of maximal proliferation by MH60.BSF2, were incubated with various concentrations of rabbit anti-rIL-6 IgG (●) or non-immune rabbit IgG (○) for 1 hr at 37°, and each neutralized sample was tested for the ability to promote the proliferation of MH60.BSF2. Results for EBV-transformed B cells are the mean ± SD of three different cell lines performed in duplicate.

background (up to 10 grains). Slides that were hybridized with sense probes gave background labelling.

Proliferative response of RPMI-1788 cells to rIL-6

RPMI-1788 cells were extensively washed with RPMI-1640 medium without FCS, adjusted to $0{\cdot}5\text{–}16 \times 10^3$ cells per well (200 μl) in culture medium and incubated for 3 days in 96-well flat-bottomed microtitre plates (Corning Glass Works) either alone or with rIL-6. Cell proliferation was measured by [^{3}H]thymidine ([^{3}H]TdR; New England Nuclear, Boston, MA) incorporation, during the 18-hr incubation, at 0·5 μCi per culture.

RESULTS

IL-6 activity in culture supernatants

IL-6 activity in culture supernatants from EBV-transformed cell lines was evaluated by the ability to promote the growth of an IL-6-dependent murine hybridoma clone, MH60.BSF2. In preliminary experiments, it was found that this hybridoma clone proliferates only in response to rIL-6, but not to other recombinant preparations of cytokines, such as IL-1, IL-2, IL-3, IL-4, interferons (IFN), or colony-stimulating factors. The detection threshold of the assay was 0·03 U/ml, which was equivalent to 6 pg/ml of rIL-6. Culture supernatants from all EBV-transformed B-cell lines examined contained detectable IL-6 activity, ranging from 0·1 to 5·0 U/ml (Fig. 1). A long-established EBV-transformed cell line, RPMI-1788, appeared to secrete much higher levels of IL-6 activity into the culture fluids than some other EBV-transformed cell lines. In contrast, no IL-6 activity was detected in the culture supernatants from Burkitt's lines such as Raji and Daudi. The addition of PMA markedly augmented the IL-6 activity in culture supernatants from EBV-transformed B cells (Fig. 2), but not from other B-cell lines (data not shown).

To examine whether IL-6 activity found in culture supernatants from EBV-transformed B cells reflected the real production of native IL-6 proteins, the neutralization experiments using rabbit anti-rIL-6 antibody were carried out (Fig. 3). The growth-promoting activity for a hybridoma clone in culture supernatants from EBV-transformed B-cell lines were almost completely abolished by the rabbit anti-rIL-6 antibody, but not by normal rabbit serum.

Western blot analysis of IL-6 in culture supernatants

Western blot analyses using the rabbit anti-rIL-6 antiserum were used to further examine whether EBV-transformed B-cell lines secreted the IL-6 molecules into the culture fluids (Fig. 4). A single band of rIL-6 of 21,000 MW was demonstrable in Western blots. Similarly, the anti-rIL-6 antiserum could detect the IL-6 proteins, presumably derived from monocytes, in culture supernatants from LPS-stimulated peripheral blood mononuclear cells (PBMC). Of importance, Western blot analyses also disclosed the immunoreactive patterns with the major band of about 23,000 MW in culture supernatants from EBV-transformed B cells, including RPMI-1788,TM-1 and HS-2. The intensity of these bands was increased after stimulation of cells with PMA. In contrast, the culture supernatants

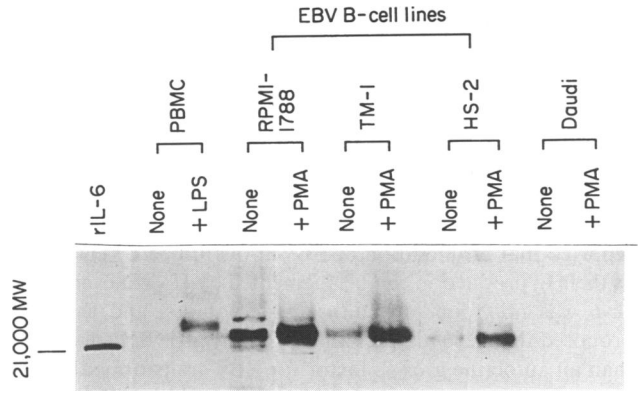

Figure 4. Western blot analyses of IL-6 proteins using a rabbit antiserum against rIL-6. EBV-transformed B-cell lines, RPMI-1788, TM-1 or HS-2, and a Burkitt's line, Daudi, were cultured with or without PMA. Lyophilized materials of culture supernatants (5 ml) were prepared as described in the Materials and Methods and used for Western blot analysis using a rabbit anti-rIL-6 antiserum. A 0·5-ml supernatant sample from unstimulated or LPS-stimulated PBMC was lyophilized and assayed similarly. The rIL-6 was included in the Western blot analysis as a control.

from a Burkitt's line, Daudi, did not contain any identifiable IL-6 proteins, even after PMA stimulation. Interestingly, heterogeneity of immunoreactive proteins was observed in culture supernatants from EBV-transformed B cells as well as LPS-stimulated PBMC.

Detection of IL-6 mRNA by *in situ* hybridization

RNA–RNA *in situ* hybridization technique with an anti-sense IL-6 probe revealed that EBV-transformed B cells expressed abundant IL-6 mRNA, although the number of grains varied from cell to cell (Fig. 5). However, when the sense RNA probe was tested, only background labelling was observed. IL-6 mRNA was expressed strongly on a fraction of EBV-transformed B cells, but on the other hand only few grains were seen on Burkitt's cell lines such as Daudi.

It is plausible to suppose that the number of grains per cell demonstrated by *in situ* hybridization may correspond to the amount of transcripts by each cell. The amount of IL-6 mRNA expressed by each cell was judged by the number of grains counted per cell. A substantial proportion (30–50%) of EBV-transformed B cells, such as RPMI-1788, HS-1 and HS-2, was considered positive for IL-6 gene expression, whereas Daudi cells were all negative. The variability of IL-6 mRNA expression on EBV-transformed B cells should be noted. In addition, it was shown that only a proportion of cells expressed IL-6 mRNA strongly.

Proliferation of EBV-transformed B cells in response to rIL-6

To evaluate the effect of IL-6 on the growth of EBV-transformed B cells, various concentrations of RPMI-1788 cells were cultured with or without rIL-6 (Fig. 6). At high cell densities, RPM1-1788 cells proliferated well without added rIL-6, and their proliferation was not augmented by the addition of rIL-6. In contrast, when RPMI-1788 were seeded at low density, the addition of rIL-6 enhanced their proliferation. Optimal growth enhancement was achieved at 0·5 U/ml rIL-6. The same results were obtained using another two lines (data not shown).

DISCUSSION

In this report, it is demonstrated that EBV-immortalized B-cell lines constitutively produced IL-6 proteins. In contrast, no IL-6

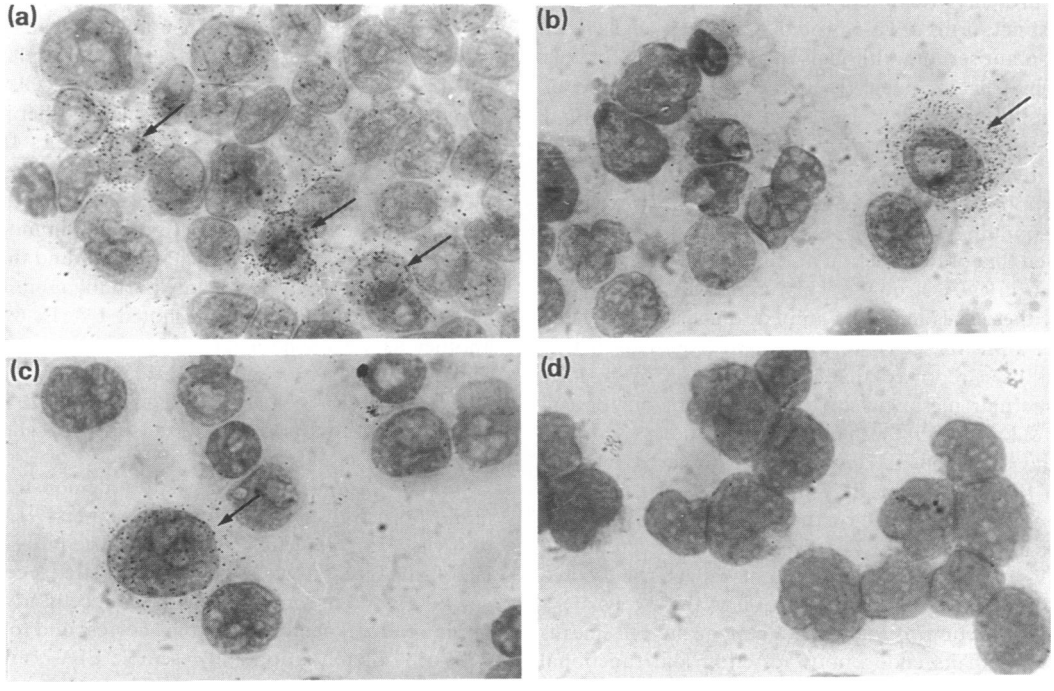

Figure 5. Detection by *in situ* hybridization of IL-6 mRNA in an EBV-transformed B-cell line RPMI-1788 (a, b, c) and a Burkitt's line, Daudi (d). It was noted that some of RPMI-1788 cells hybridized obviously (arrows), but Daudi cells did not.

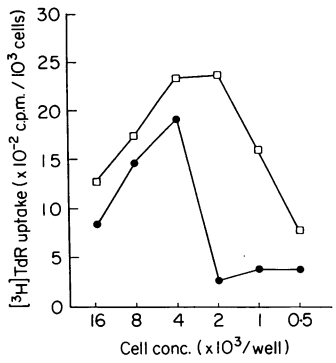

Figure 6. Growth of RPMI-1788 cells by rIL-6. RPMI-1788 cells were seeded at varying numbers in a volume of 200 μl per well with (□) or without (●) 0·5 U/ml of rIL-6. DNA synthesis at 3 days was assessed by pulsing wells with 0·5 μCi of [^{3}H]TdR for the final 18 hr. The results were expressed as arithmetic mean c.p.m. of triplicate cultures.

activity was observed in culture supernatants from other EBV-negative B-cell lines. In addition, it was shown that Burkitt's lines, Daudi or Raji, did not produce any IL-6 activity into the culture fluids, although they carry the EBV-genome, as do EBV-transformed B cells. The basis of this discrepancy for IL-6 production between both EBV-carrying B cells is unknown.

Western blot analysis also confirmed that supernatants from cultured EBV-transformed B cells contained several immunoreactive forms of IL-6, with a major species of 23,000 MW. IL-6 production has been described in a variety type of cells, including fibroblasts, monocytes and endothelial cells (May *et al.*, 1988; Van Damme *et al.*, 1987; Yang *et al.*, 1988; Sironi *et al.*, 1989; Van Snick *et al.*, 1987). It has been shown that human fibroblasts and monocytes synthesize and secrete several distinct forms of IL-6 proteins with different molecular sizes (May *et al.*, 1988). The distinct forms of IL-6 proteins in different cells react with anti-rIL-6 antiserum. Although the exact reason for this molecular weight heterogeneity is unknown, the secretion of multiple forms of IL-6 might be associated with differential glycosylation.

By *in situ* hybridization, the presence of IL-6 mRNA in a substantial proportion of EBV-transformed B cells was demonstrated corresponding with their secretion of IL-6 proteins. It should be noted that only a fraction of EBV-transformed B cells expressed IL-6 mRNA strongly. This implies that IL-6 gene expression by these cells might be limited to a specific stage of the cell cycle, since the cells were not synchronized. In this regard, it has been reported that the production of IL-1 by cultured glomerular mesangial cells is dependent on the cell cycle (Lovett & Larsen, 1988). In unpublished studies (K. Kato, A. Yachie and T. Miyawaki), it has been observed that the expression of IL-6 mRNA by EBV-immortalized B cells could be markedly augmented by the treatment with hydroxyurea (S phase-specific agent). It seemed that relatively low secretion of IL-6 by EBV-transformed B cells might reflect the limited expression of IL-6 mRNA by a proportion of these cells.

The autocrine action of a growth factor in the cell lines is explainable by both its endogenous secretion and functional receptor expression by the cells. In the present study, it is shown that EBV-transformed B cells produced IL-6 in culture and the addition of rIL-6 resulted in the appreciable growth of EBV-transformed B cells at low cell densities. The proliferation of EBV-transformed B cells was enhanced by a small amount (less than 0·5 U/ml or 100 pg/ml) of rIL-6. The ability of EBV-transformed B cells to produce IL-6, though relatively low, seemed to be sufficient to maintain the growth of these cells, especially at high cell densities. Recently, the receptors for IL-6 has been identified on many EBV-transformed B cells but not on Burkitt's lines (Taga *et al.*, 1987). Tosato *et al.* (1988a, b) has reported that proliferation of EBV-transformed B cells as well as their Ig production can be augmented by IL-6. Nevertheless, IL-6 was not found in culture supernatants, and they thus proposed that IL-6 may act as a paracrine growth factor rather than an autocrine growth factor for EBV-transformed B cells.

With respect to the autocrine growth role of IL-6, it has recently been suggested that the expression of IL-6 and its receptor could be responsible for the oncogenic generation of human multiple myeloma (Kawano *et al.*, 1988). It has recently been reported that the responsiveness of myeloma cells to IL-6 decreases with the progression of disease stage (Asaoku *et al.*, 1988). These observations are very interesting, considering the difference in the autocrine growth role of IL-6 between EBV-transformed B cells and Burkitt's lymphoma cells. The transformation of Burkitt's lymphoma cells might be a result of down-regulation of expression of IL-6 and its receptor.

IL-1, IL-4, IFN-γ and lymphotoxin as well as IL-6, have been detected in culture supernatants from the EBV-transformed B-cell line RPMI-1788. However, only anti-IL-1α antibody abolished the autocrine growth activity seen in RPMI-1788, indicating that IL-1α might be the only autocrine growth factor in this cell line (Vandevabeele *et al.*, 1988). It is well known that IL-1 can induce or promote the production of IL-6 by many cells, such as fibroblasts, endothelial cells (May *et al.*, 1988; Van Damme *et al.*, 1987; Yang *et al.*, 1988; Sironi *et al.*, 1989) and monocytes (K. Kato and T. Miyawaki, unpublished observations). Although there is a possibility that IL-1 could modulate the expression of IL-6 in EBV-transformed B cells, resulting in the promotion of growth, the interplay between IL-6 and IL-1 in the autocrine growth of these cells remains to be elucidated. Besides IL-1α or IL-6, several other molecules have been proposed as autocrine growth factors for EBV-transformed B-cell lines (Gordon *et al.*, 1984; Wakasugi *et al.*, 1987; Swendeman & Thorley-Lawson, 1987). Thus, the mechanisms involved in the autocrine growth of EBV-immortalized B cells are not straight forward. In fact, it was found that excess doses of anti-IL-6 antibody could only partially inhibit the growth of EBV-transformed B cells promoted by rIL-6. Furthermore, another as yet uncharacterized growth factor with low molecular weight has recently been identified in supernatants of EBV-transformed cell lines (Tosato, Tanner & Pike, 1989).
fied in supernatants of EBV-transformed cell lines (Tosato, Tanner & Pike, 1989).

In conclusion, the present study demonstrates that EBV-immortalized B cells constitutively express IL-6 protein and mRNA. IL-6 gene expression has been observed in fresh neoplastic B cells as well as normal activated B cells (Freeman *et al.*, 1989), and in some of B lymphoma cells, myeloma cells, or plasma cell leukaemia, which may correspond to the later stages of B-cell differentiation and resemble EBV-transformed pre-B cells (Kubagawa *et al.*, 1989). These observations imply a biologically important role for B cell-derived IL-6. cDNA encoding putative human IL-6 receptors has been isolated

(Yamasaki *et al.*, 1988). Further studies will be needed to elucidate how the production of IL-6 or its receptor expression by human B cells are regulated in their differentiation process.

ACKNOWLEDGMENTS

We thank Drs T. Hirano and T. Kishimoto (Osaka University) for kindly providing us with reagents and cells, Dr L.T. May (Rockefeller University) for anti-rIL-6 antiserum, and Dr K. Yamamoto (Kanazawa University) for normal rabbit IgG. We also appreciate the valuable advice of Drs H. Sato (Kanazawa University), G. Borzillo (St Jude Children's Hospital) and M. D. Cooper (University of Alabama) and thank Misses R. Miyazaki and K. Nakajima for their excellent technical assistance.

This research was supported by Grant-in-Aids for Scientific Research from the Ministry of Education in Japan, and grants from the Ministry of Welfare in Japan, from Uehara Memorial Foundation and from Mother-and Child's Health Foundation.

REFERENCES

ASAOKU H., KAWANO M., IWATO K., TANABE O., TANAKA H., HIRANO T., KISHIMOTO T. & KURAMOTO A. (1988) Decrease in BSF-2/IL-6 response in advanced cases of multiple myeloma. *Blood*, **72,** 429.

BLAZAR B.A., SUTTON L.M. & STROME M. (1983) Self-stimulating growth factor production by B cell lines derived from Burkitt's lymphomas and other lines transformed *in vitro* by Epstein–Barr virus. *Cancer Res.* **43,** 4562.

FREEMAN G.J., FREEDMAN A.S., RABINOWE S.N., SEGIL J.M., HOROWITZ J., ROSEN K., WHITMAN J.F. & NADLER L.M. (1989) Interleukin 6 gene expression in normal and neoplastic B cells. *J. clin. Invest.* **83,** 1512.

GORDON J., LEY S.C., MELAMED M.D., ENGLISH L.S. & HUGHES-JONES N.C. (1984) Immortalized B lymphocytes produce B-cell growth factor. *Nature (Lond)*, **310,** 145.

HIRANO T., YASUKAWA K., HARADA H., TAGA T., WATANABE Y., MATSUDA T. *et al.* (1986) Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature (Lond.)*, **324,** 73.

KATO K., YOKOI T., TAKANO N., KANEGANE H., YACHIE A., MIYAWAKI T. & TANIGUCHI N. (1990) Detection by *in situ* hybridization and phenotypic characterization of cells expressing IL-6 mRNA in human stimulated blood. *J. Immunol.* (in press).

KAWANO M., HIRANO T., MATSUDA T., TAGA T., HORII Y., IWATO K. *et al.* (1988) Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. *Nature (Lond.)*, **332,** 83.

KUBAGAWA H., BURROWS P.D., GROSSI C.E., MESTECKY J. & COOPER M. D. (1988) Precursor B cells transformed by Epstein–Barr virus undergo sterile plasma-cell differentiation: J-chain expression without immunoglobulin. *Proc. natl. Acad. Sci. U.S.A.* **85,** 875.

KUBAGAWA H., COOPER M.D., CARROLL A.J. & BURROWS P.D. (1989) Light-chain gene expression before heavy-chain gene rearrangement in pre-B cells transformed by Epstein-Barr virus. *Proc. natl. Acad. Sci U.S.A.* **86,** 2356.

LOVETT D.H. & LARSEN A. (1988) Cell cycle-dependent interleukin 1 gene expression by cultured glomerular mesangial cells. *J. clin. Invest.* **82,** 115.

MATSUDA T., HIRANO T. & KISHIMOTO T. (1988) Establishment of interleukin 6 (IL-6)/B cell stimulatory factor 2-dependent cell line and preparation of anti-IL 6 monoclonal antibodies. *Eur. J. Immunol.* **18,** 951.

MAY L.T., GHRAYEB J., SANTHANAM U., TATTER S.B., STHOEGER Z., HELFGOTT D.C., CHIORAZZI N., GRIENINGER G. & SEHGAL P.B. (1988) Synthesis and secretion of multiple forms of β2-interferon/B-cell differentiation factor 2/hepatocyte-stimulating factor by human fibroblasts and monocytes. *J. Biol. Chem.* **263,** 7760.

MIYAWAKI T., KUBAGAWA H., BUTLER J.L. & COOPER M.D. (1988) Ig isotypes produced by EBV-transformed B cells as a function of age and tissue distribution. *J. Immunol.* **140,** 3887.

SIRONI M., BREVIARIO F., PROSERPIO P., BIONDI A., VECCHI A., VAN DAMME J., DEJANA E. & MANTOVANI A. (1989) IL-1 stimulates IL-6 production in endothelial cells. *J. Immunol.* **142,** 549.

SPORN M.B. & ROBERTS A.B. (1985) Autocrine growth factors and cancer. *Nature (Lond.)*, **313,** 745.

SWENDEMAN S. & THORLEY-LAWSON D.A. (1987) The activation antigen Blast-2, when shed, is an autocrine BCGF for normal and transformed B cells. *EMBO J.* **6,** 1637.

TAGA T., KAWANISHI Y., HARDY R.R., HIRANO T. & KISHIMOTO T. (1987) Receptors for B cell stimulatory factor 2: quantitation, specificity, distribution, and regulation of their expression. *J. exp. Med.* **166,** 967.

THORLEY-LAWSON D.A. (1988) Basic virological aspects of Epstein–Barr virus infection. *Sem. Hematol.* **25,** 247.

TOSATO G. & BLAESE R.M. (1985) Epstein–Barr virus infection and immunoregulation in man. *Adv. Immunol.* **37,** 99.

TOSATO G., GERRARD T.L., GOLDMAN N.G. & PIKE S.E. (1988a) Stimulation of EBV-activated human B cells by monocytes and monocyte products. *J. Immunol.* **140,** 4329.

TOSATO G., SEAMON K.B., GOLDMAN N.D. SEHGAL P.B., MAY L.T., WASHINGTON G.C., JONES K.D. & PIKE S.E. (1988b) Monocyte-derived human B-cell growth factor identified as interferon-β_2 (BSF-2, IL-6). *Science*, **239,** 502.

TOSATO G., TANNER J. & PIKE S.E. (1989) Identification of a low molecular weight B cell growth factor in the supernatant of a lymphoblastoid B cell line. *FASEB J.* **3,** A497.

UENO Y., TAKANO N., KANEGANE H., YOKOI T., YACHIE A., MIYAWAKI T. & TANIGUCHI N. (1989) The acute phase nature of interleukin 6: studies in Kawasaki disease and other febril illnesses. *Clin. exp. Immunol.* **76,** 337.

VAN DAMME J., OPDENAKKER G., SIMPSON R.J., RUBIRA M.R., CAYPHAS S., VINK A., BILLIAU A. & VAN SNICK J. (1987) Identification of the human 26-kD protein, interferon β_2 (IFN-β_2), as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumour necrosis factor. *J. exp. Med* **165,** 914.

VANDEVABEELE P., JAYARAM B., DEVOS R., SHAW A. & FIERS W. (1988) Interleukin 1α acts as an autocrine growth factor for RPMI 1788, an Epstein–Barr virus-transformed human B cell line. *Eur. J. Immunol.* **18,** 1027.

VAN SNICK J., VINK, A., CAYPHAS S. & UYTTENHOVE C. (1987) Interleukin-HP1, a T cell-derived hybridoma growth factor that supports the in vitro growth of murine plasmacytomas. *J. exp. Med.* **165,** 641.

WAKASUGI H., RIMSKY L., MAHE Y., KAMEL A.M., FRADELIZI D., TURSZ T. & BERTOGLIO J. (1987) Epstein–Barr virus-containing B-cell line produces an interleukin 1 that it uses as a growth factor. *Proc. natl. Acad. Sci. U.S.A.* **84,** 804.

YAMASAKI K., TAGA T., HIRATA Y., YAWATA H., KAWANISHI Y., SEED B., TANIGUCHI T., HIRANO T. & KISHIMOTO T. (1988) Cloning and expression of the human interleukin-6 (BSF-2/IFNβ 2) receptor. *Science*, **241,** 825.

YANG Y.-C., TSAI S., WONG G.G. & CLARK S.C. (1988) Interleukin-1 regulation of hematopoietic growth factor production by human stromal fibroblasts. *J. Cell. Physiol.* **134,** 292.