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Immunology

Epstein–Barr virus-containing B-cell line produces an interleukin 1 that it uses as a growth factor

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ABSTRACT **We report the establishment of a spontaneous interleukin 1 (IL-1)-producing subclone derived from the human Epstein–Barr virus (EBV)-containing B-lymphoblastoid cell line (721 LCL) and show that the IL-1 produced by this B-cell subclone is distinct from other types of IL-1. The parental cell line 84.5, a deletion mutant of the 721 LCL cell line, can be induced to produce IL-1 activity when stimulated by certain inducers such as phorbol 12-myristate 13-acetate in the presence of fetal calf serum. From this parental 721/84.5 clone, a subclone, termed 3B6, has been developed. This 3B6 subclone has an immature B-cell phenotype, expresses only HLA class II DP subregion antigens, and spontaneously releases IL-1 in the culture supernatant with relatively few inhibitory molecules under serum-free culture conditions. The 3B6-derived IL-1 was purified from 3B6 conditioned medium with a three-step procedure. The molecular weight of this IL-1 is 13,500, and the isoelectric point values are pH 4.9 and 5.1 without any component focusing near pH 7. The N-terminal amino acid sequence differs markedly from those reported for the two IL-1 species produced by monocytes. The purified material shares several biological properties with monocyte IL-1, since it could induce (*i*) the proliferation of murine thymocytes, (*ii*) the production of interleukin 2 by phytohemagglutinin-stimulated cloned HSB2 T cells, and (*iii*) the proliferation of human fibroblasts. However, this IL-1 activity could not be blocked by polyclonal anti-monocytic IL-1 antibodies, and, more importantly, it was not pyrogenic in rabbits. Finally, it promotes the growth of B-cell clones derived from the parental 721/84.5 lines in the absence of fetal calf serum, which suggests that it could act as an autocrine growth factor in this Epstein–Barr virus-transformed B-cell line.**

Stimulated monocytes have been shown to produce a monokine named interleukin 1 (IL-1) that plays a central role in T-cell activation. It can promote thymocyte proliferation in the presence of phytohemagglutinin (PHA) and induces the secretion of interleukin 2 (IL-2) by various T-cell lines (1, 2). It is now agreed that IL-1 is identical to the lymphocyte activating factor (3). Furthermore, numerous other biological properties have been assigned to IL-1, which suggests that this monokine plays a role in the acute phase of the inflammatory process (4). In particular, IL-1 could sustain the proliferation of fibroblasts (5) and is pyrogenic *in vivo* (6); the identity between IL-1 and endogenous pyrogen has been supported by several experimental observations.

It is now believed that IL-1s form a family of molecules. Monocytes apparently produce two prominent species, with molecular weights reported between 12,000 and 17,000. The major species (IL-1β) exhibits a pI value of 7. Two different groups have cloned cDNAs corresponding to the mRNA coding for IL-1β and have reported very similar sequences (7, 8). Two additional independent publications have reported the N-terminal amino acid sequences of a 17-kDa IL-1 and of a 22-kDa γ-interferon-inducing factor, which show large homology with the amino acid sequence deduced from these IL-1β cDNA sequences (9, 10).

A minor species of human IL-1 (IL-1α) produced by stimulated monocytes has a similar molecular weight, but a more acidic pI value, around 5. A human cDNA sequence that could code for IL-1α has been reported (8). Homologous cDNA sequences have been reported in mice (11) and in rabbits (12).

It is now clear that IL-1 production is not restricted to monocytes. For example, keratinocytes, glial cells, and B cells have been shown to produce IL-1 (13–16). However, these cell sources have not been able to provide enough material for biochemical characterization. Human cell lines of nonmonocytic origin producing large quantities of IL-1 would be valuable tools for the characterization of other types of IL-1 and their comparison with the monocytic IL-1s already described.

We present here the derivation and characterization of a subclone derived from an Epstein–Barr virus-(EBV) infected B-cell line that (*i*) expresses HLA-DP antigens in the absence of detectable HLA-DR and -DQ antigens, (*ii*) spontaneously produces high amounts of IL-1, (*iii*) requires IL-1 for its growth under serum-free culture conditions. The IL-1-like material produced by this B-cell clone has been purified to homogeneity (17) and has a molecular weight of 13,500 and pI values of 4.9 and 5.1. As reported in detail elsewhere, the N-terminal amino acid sequence of this B-cell line-derived IL-1 is not homologous with any sequence reported for IL-1α or -1β or other lymphokines. This IL-1 promotes the proliferation of murine thymocytes and fibroblasts, induces the production of IL-2 by the cloned HSB2 line, but differs from monocytic IL-1 since it cannot be blocked by polyclonal anti-monocytic IL-1 antibody and appears devoid of pyrogenic activity.

MATERIALS AND METHODS

Cell Lines. The human B-cell line used in this study derived from a lymphoblastoid cell line (LCL), termed 721/84.5. This cell line was mutagenically derived from its parental line LCL 721 in the presence of anti-HLA-DR monoclonal antibodies and complement. The mutant 721/84.5 lacks one part of chromosome 6 and was reported not to express class II major histocompatibility complex antigens (18). The 721 and 721/84.5 LCL cells were kindly given to us by R. DeMars (University of Wisconsin). These cell lines were routinely cultured in RPMI 1640 medium supplemented with 10%

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Abbreviations: ATL, adult T-cell leukemia; EBV, Epstein-Barr virus; FCS, fetal calf serum; IL-1, interleukin 1; IL-2, interleukin 2; PMA, phorbol 12-myristate 13-acetate; PHA, phytohemagglutinin; ADF, ATL-derived factor.

(vol/vol) fetal calf serum (FCS; Seromed, Mulhouse, France).

Antibodies. Polyclonal anti-monocytic IL-1 antisera was kindly provided by C. A. Dinarello (Tufts University School of Medicine, Boston) (19).

Cloning of IL-1-Producing Cell Line. From the 721/84.5 mutant line, a subclone termed 3B6 has been developed. The 721/84.5 cells were cultured at 0.5 cell per well in the routine culture medium (RCM) consisting of RPMI 1640 (Gibco) supplemented with 15% (vol/vol) heat-inactivated FCS, additional 2 mM L-glutamine, and gentamycin (20 μg/ml) in 96-well microculture plates (Falcon). Two weeks after the start of cloning, the growing subclones were transferred to plastic culture flasks (Falcon), were phenotyped, and tested for their IL-1-producing ability. The most interesting subclones have been karyotyped to confirm their origin.

Lymphokines. For IL-1 preparation, the 3B6 cells were grown in RCM, collected, extensively washed to remove the residual FCS, adjusted to 2×10^6 cells per ml, and further incubated for 3 days in serum-free culture conditions. The supernatants were collected by successive centrifugation at $800 \times g$ for 10 min at room temperature, followed by 10,000 $\times g$ for 1 hr at 4°C, and stored at 4°C with sodium azide (0.04%, final concentration) until further use. In some experiments, the cells were incubated with phorbol 12-myristate 13-acetate (PMA) (1 μg/ml) in serum-free medium. Highly purified monocyte IL-1 was kindly provided by C. A. Dinarello.

IL-1 Assay. *Thymocyte proliferation assay.* IL-1 activity was measured in the C3H/Hej mouse thymocyte comitogenic assay (20). IL-1 activity was calculated from the linear portion of the [^{3}H]thymidine ([^{3}H]dThd) incorporation data by a method similar to the procedure used for determining IL-2 activity (21). Each sample tested for IL-1 activity was compared with the highly purified monocyte IL-1 as standard. Functionally this preparation gave 50% of the maximum proliferation in the thymocyte proliferation assay at the 1:32 dilution. In the inhibition experiments, either 3B6 IL-1 or monocyte IL-1 was incubated with PHA (10 μg/ml)-stimulated murine thymocytes (1.5×10^6 cells per well) in the presence of polyclonal anti-IL-1 sera (final concentration, 1%) for 3 days in microplate wells (Falcon). [^{3}H]dThd incorporation was measured by the addition of 1 μCi of [^{3}H]dThd (1 Ci = 37 GBq) during the last 4 hr of culture in duplicate samples.

Fibroblast proliferation assay. Cells from the HSF 809 fibroblast were seeded at 5000 per well in microculture plates in Dulbecco's minimal essential medium (DMEM) supplemented with serial dilutions of the test sample as described (5). The cultures were pulsed with [^{3}H]dThd for the last 18 hr of the 3-day incubation.

Production of IL-2 by HSB2 cells. Similarly to Kasahara *et al.* (22), we have cloned the HSB2 line and obtained several subclones. One of the subclones, termed 8G5, produces large amounts of IL-2 only in the presence of PHA and IL-1. 8G5 cells were adjusted to 10^5 cells per well and incubated in the microculture plates either in the presence of IL-1 preparation and/or PHA (50 μg/ml). Twenty-four hours later, the supernatants were collected, filter sterilized, and assayed for IL-2 activity. For the IL-2 assay, murine IL-2-dependent cytotoxic T cells (CTLL 2) were used as described (23).

Growth of Subclone 3B6 in the Presence of IL-1. The 3B6 cells were extensively washed with RCM without FCS, adjusted then to 10^4–10^5 cells per well using RCM supplemented with 0–5% (vol/vol) FCS, and incubated for 3 days in the flat-bottomed microculture plates either in the presence or absence of 3B6 IL-1. The effect of monocyte IL-1 was assessed in the same way. Thymidine incorporation was measured by the addition of 1 μCi of [^{3}H]dThd during the last 4 hr of triplicated culture.

Biochemical Characterization of IL-1 Material. Detailed procedures for the purification of IL-1 have been described (17). Briefly, the purification procedure included a sequence of SP-Sephadex ion-exchange chromatography, fast protein liquid chromatography (FPLC), HR5/5 MonoQ ion-exchange chromatography, and an affinity chromatography on procion red agarose.

Analytical chromatofocusing. Samples of crude supernatant from the 3B6 line were analyzed by chromatofocusing on an FPLC Mono-P HR 5/20 column (Pharmacia) as described (17). Fractions of 1 ml were collected and assayed for biological activity.

Radiolabeling. For radiolabeling, Iodo-Beads (Pierce) were incubated with 100 μl of 50 mM potassium phosphate, pH 7.0, and 0.1 mCi of ^{125}I (New England Nuclear) for 5 min. Purified IL-1 (10 μl) was added to this radioactive preparation and incubated on ice for 15 min. Free ^{125}I was separated with a PD10 column (Pharmacia) that had been equilibrated in the same buffer. The sample was concentrated by a factor of 10 by evaporation on a Speed Vac system (Savant) and analyzed by NaDodSO$_4$/PAGE [7–15% (wt/vol) acrylamide gradient]. The gel obtained was dried and exposed overnight with an x-ray film (XAR-5; Kodak).

RESULTS

Establishment of the Spontaneous IL-1-Producing Subclones. Results are presented in Fig. 1. About 60 subclones were obtained from 100 wells and tested for their ability to produce IL-1. Among these 60 clones, two clones, 3B6 and 4E5, spontaneously produced 0.3–0.5 unit of IL-1 activity without any exogenous stimulation (Fig. 1). Their IL-1 production was increased by stimulation with PMA (1 μg/ml) (Fig. 2). No stimulation of IL-1 production was observed when these clones were incubated in the presence of FCS (data not shown). This constitutive production of IL-1 has been very stable since these two clones have continuously produced IL-1 activity for more than 18 months. By contrast, another clone, termed 2E5, did not produce IL-1 activity even when stimulated by PMA (Figs. 1 and 2).

Analytical Chromatofocusing. When concentrated, crude supernatant from 3B6 cells, containing about 10 units of IL-1 activity, was applied to an FPLC Mono-P HR 5/20 chromatofocusing column, and IL-1 activity was eluted in two separate peaks corresponding to pI values of 5.1 and 4.9

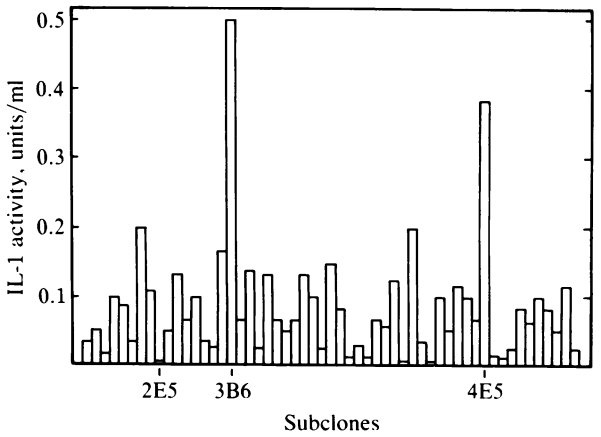

FIG. 1. IL-1 production by 721/84.5 subclones. About 60 subcloned cells from the 721/84.5 line were maintained in RCM, then collected, extensively washed, adjusted to 2×10^6 cells per ml, and further incubated for 3 days in the serum-free culture condition. The supernatants were collected, filter sterilized, and assayed for their IL-1 activity assessed by PHA-stimulated murine thymocyte proliferation.

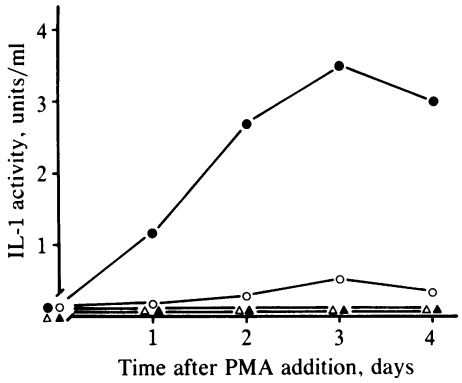

FIG. 2. Kinetics of IL-1 production by 721/84.5 subclones. 3B6 or 2E5 cells were extensively washed, adjusted to 2×10^6 cells per ml, and further incubated for 1–4 days either in the presence or absence of PMA (1 μg/ml) in the serum-free culture condition. The conditioned media were collected and assayed for their IL-1 activity. "Days" indicates the day of media collection after addition of PMA. When cells have been triggered with PMA, the background due to PMA alone has been subtracted from IL-1 units. Results represent mean of duplicate cultures of a reproducible experiment. ○, 3B6 cells; ●, PMA-treated 3B6 cells; △, 2E5 cells; ▲, PMA-treated 2E5 cells.

(Fig. 3). No component with a pI value of 7 could be detected (see ref. 17).

Purity and Molecular Weight of B-Cell-Derived IL-1. In Fig. 4, an autoradiogram of ^{125}I-radiolabeled, purified IL-1 is presented. The IL-1 migrated as a single band of 13.5 kDa and was free of any detectable protein contaminants. IL-1 was recovered as a homogenous protein with apparent purity of more than 99.9% with a yield of 60% (see ref. 17). No detectable IL-2 activity was observed at any step of the purification.

Biological Properties of Purified IL-1. In Fig. 5, various biological properties of the purified IL-1 are displayed and compared with those of highly purified monocyte IL-1. The 3B6-derived IL-1 very efficiently promoted the proliferation of PHA-stimulated murine thymocytes (Fig. 5*A*). The 3B6 IL-1 could promote the proliferation of fibroblasts (Fig. 5*B*).

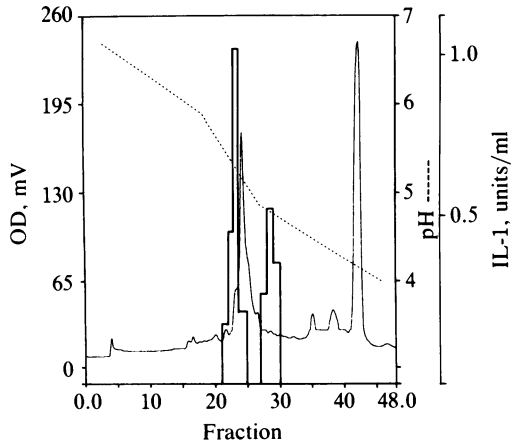

FIG. 3. Chromatofocusing analysis of crude 3B6 IL-1. Concentrated crude conditioned medium (5–10 units) was diluted with the starting buffer containing 25 mM imidazole·HCl, pH 7.4, supplemented with 10% (vol/vol) acetonitrile and applied to the chromatofocusing column. After washing with the starting buffer, IL-1 was eluted with Polybuffer 74, pH 4.0, supplemented with 10% (vol/vol) acetonitrile. Fractions (1 ml) were collected and assayed for their IL-1 activity by the proliferation of PHA-stimulated murine thymocyte (bold lines). OD at 280 nm is shown by the solid line; 1.0 = 500 mV.

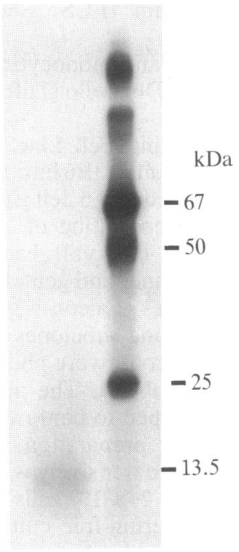

FIG. 4. Molecular weight of B-cell-derived IL-1. Purified IL-1 (10 μl) was incubated with one Iodo-Bead and ^{125}I for 15 min, dialyzed against potassium phosphate, evaporated, concentrated, and analyzed by $NaDodSO_4$/PAGE [7–15% (vol/vol) acrylamide gradient]. The gel obtained was dried and exposed to x-ray film.

The 3B6-IL-1 titration curve in this assay is very similar to that of purified monocyte IL-1. Finally, 3B6 IL-1 was able to induce the production of IL-2 by cloned HSB2 line (Fig. 5*C*). Again, 3B6 IL-1 was as effective as monocyte IL-1 in this assay (Fig. 5*C*). However, 3B6 IL-1 was not pyrogenic in rabbits up to 3 ng/kg of the IL-1 material using the intracerebroventricular, direct-injection method that corresponds to 1500 ng/kg by the usual intravenous injection method.

Inhibition of IL-1 Activity by Anti-IL-1 Antisera. The polyclonal anti-monocyte IL-1 that is known to inhibit at both type α and β monocyte IL-1 effectively suppressed the activity of monocyte-derived IL-1 (Fig. 6*A*) but did not inhibit 3B6-IL-1 activity (Fig. 6*B*). These results suggest that the B-cell-derived IL-1 does not share epitopes recognized by

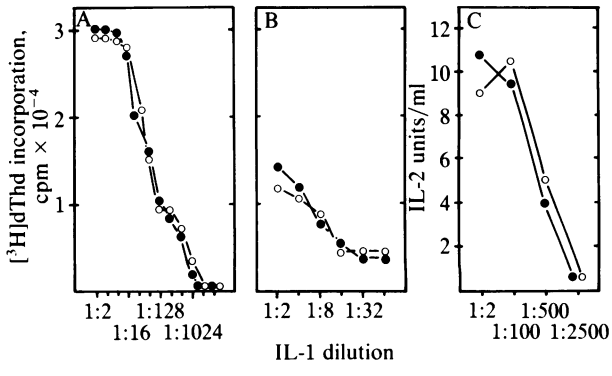

FIG. 5. Biological properties of 3B6 IL-1. (*A*) IL-1 activity was titrated using PHA-stimulated C3H/HeJ murine thymocytes and serially diluted 3B6 IL-1 (●) or monocyte IL-1 (○). One biological unit of IL-1 was defined as the quantity of material required to induce 50% of the maximum [^{3}H]dThd incorporation. (*B*) HSF 809 human fibroblasts were seeded at 5000 cells per well in microtiter plates in DMEM with serially diluted 3B6 IL-1 (0.1 unit/ml) (●) or monocyte IL-1 (0.1 unit/ml) (○). The cultures were pulsed with [^{3}H]dThd for the 18 hr of a 3-day incubation. (*C*) Cloned HSB2 cells, termed 8G5 cells, were adjusted to 10^5 cells per well in RCM and incubated in the presence of IL-1 preparations (●, 3B6 IL-1, or ○, monocyte IL-1, at 0.1 unit/ml) and/or PHA (50 μg/ml) for 24 hr. The collected conditioned media were assayed for their IL-2 activity using CTLL 2 cells.

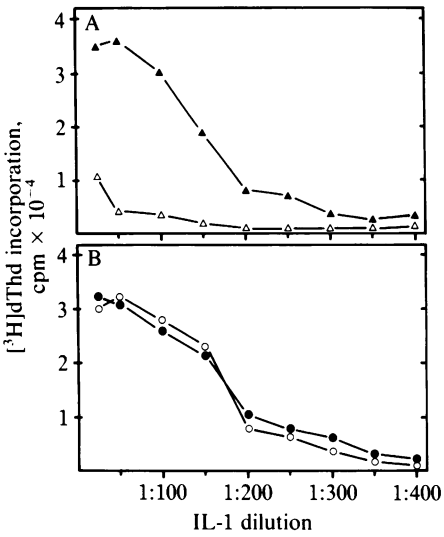

FIG. 6. Inhibition of IL-1 activity by anti-IL-1 sera. 3B6 IL-1 or monocyte IL-1 was serially diluted and preincubated with anti-IL-1 sera (1% of final volume) for 90 min at 37°C. Then, PHA-stimulated thymocytes (1.5×10^6 cells per ml) were added to the culture and incubated for 3 days in microculture plates. Thymidine incorporation was measured by adding 1 μCi of [^{3}H]dThd during the last 4 hr. Results represent mean of duplicate cultures of a reproducible experiment. (*A*) ▲, Monocyte IL-1; △, monocyte IL-1 and anti-IL-1 antisera. (*B*) ●, 3B6 IL-1; ○, 3B6 IL-1 and anti-IL-1 antisera.

this antibody raised against monocyte-derived IL-1 molecules. Similarly, anti-IL-1 sera completely inhibited the production of IL-2 induced by monocyte IL-1 on HSB2 cloned cells, whereas it left completely unaffected the same IL-2 production induced by 3B6 IL-1 (data not shown).

Effect of IL-1 on 3B6 Growth. The effects of purified IL-1 on 3B6 growth are detailed in Fig. 7. Fig. 7*A* indicates that growth of 3B6 cells was dependent upon the presence of FCS in the medium. The cells, however, could be partially relaxed from serum requirements by the addition of purified IL-1. In the complete absence of FCS (Fig. 7*B*), it was observed that only cells seeded at the highest concentration (10^5 cells per

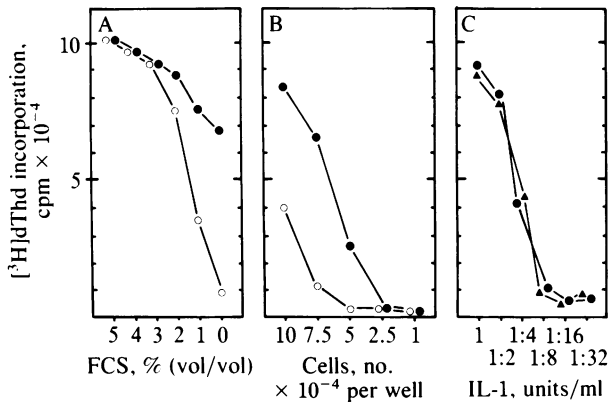

FIG. 7. Growth of 3B6 subclone in the presence of IL-1. (*A*) 3B6 cells were extensively washed with serum-free RCM, adjusted to 7.5×10^4 cells per well, and cultured in RCM supplemented with 0–5% (vol/vol) FCS for 3 days either in the presence (●) or absence (○) of 3B6 IL-1 (1 unit/ml). (*B*) The 3B6 cells were washed with serum-free RCM, adjusted to 10^4–10^5 cells per well in serum-free RCM, and incubated for 3 days either in the presence (●) or absence (○) of 3B6 IL-1 (1 unit/ml). (*C*) The 3B6 cells were washed with serum-free RCM, adjusted to 7.5×10^4 cells per well in serum-free RCM, and incubated for 3 days either in the presence of 3B6 IL-1 (1 unit/ml) (●) or monocyte IL-1 (1 unit/ml) (△). Thymidine incorporation was measured by adding 1 μCi of [^{3}H]dThd during the last 4 hr. Results represent mean of duplicate cultures of a reproducible experiment.

well) could proliferate in a significant manner. Cell proliferation was not directly proportional to the number of cells added per well, suggesting that an endogenously produced growth factor may be involved in proliferation. Indeed, addition of purified 3B6-derived IL-1 at 1 unit/ml resulted in a linear relationship between cell concentration and proliferative capacity.

In Fig. 7*C*, it is shown that monocyte IL-1 was as efficient as 3B6 IL-1 in restoring cellular growth of 3B6 cells maintained at low cellular concentration in serum-free medium. In addition, the growth of clone 2E5, which did not produce detectable amounts of IL-1, could also be enhanced under the same conditions by 3B6-derived IL-1.

DISCUSSION

We report here the characterization of a subclone of an EBV-containing B-cell line that constitutively produces significant amounts of IL-1. The isolation of this B-cell subclone allowed, to our knowledge, for the first time the purification to homogeneity of IL-1 from cells of nonmonocytic origin.

The 721 EBV-positive B-cell line was established by R. DeMars and co-workers (18) from normal B cells. He then generated the 721/84.5 line, which has been reported to lack HLA class II antigen expression and to present a deletion of the short arm of a unique chromosome 6. We have investigated the accessory function of various human leukemic cell lines (24, 25) and showed that various human B-cell lines, even when apparently lacking class II major histocompatibility complex antigens, could efficiently restore the proliferation of highly purified T cells in the presence of PHA (26). Among these lines, the 721/84.5 appeared to be a very good accessory cell and produced significant amounts of IL-1, clearly detectable after triggering with PMA. This cell line has been cloned by limiting dilution, and a clone, termed 3B6 clone, was selected for further investigation, because of its ability to produce substantial IL-1 activity without any exogenous stimulation (Fig. 1). From the same experiments, it was possible to obtain various subclones differing in their ability to produce IL-1, including the 2E5 clone that did not release IL-1 even after triggering with PMA. The 3B6 subclone expressed the same B-cell markers as the parental line (data not shown). The most striking phenotypic feature of these B-cell subclones was the absence of reactivity with a panel of monoclonal antibodies directed against the products of the HLA-DR and -DQ regions, contrasting with their apparent expression of HLA-DP antigens.

The 3B6-derived IL-1 has been purified to homogeneity. The biochemical procedures are detailed elsewhere (17). By $NaDodSO_4$/PAGE and autoradiography (Fig. 5), this IL-1 appeared as a single band at 13.5 kDa. Some degree of microheterogeneity was detected by analytical chromatofocusing with two peaks having pI values of 4.9 and 5.1 in the crude supernatants (Fig. 3). In contrast to monocyte IL-1, no IL-1 activity in the pH 7 range could be detected at any step of the purification (data not shown).

The production of IL-1 by EBV-containing B cells (27) and by normal B cells (16) has been reported. In particular, Matsushima *et al.* (29) have reported the purification of IL-1 like material in B-cell supernatants. They frequently found IL-1 inhibitors in such supernatants, i.e., contra-IL-1 (28). Upon partial purification of IL-1 from the EBV-positive B-cell line, they identified the activity in a molecule with M_r of 25,000 and with a pI value of 5.5, suggesting that B-cell-derived IL-1 could be similar to the most acidic form of monocyte IL-1 (29).

The IL-1 produced by the 3B6 clones offers a series of similarities with other IL-1 species. Biologically, it could very efficiently promote the proliferation of both PHA-stimulated murine thymocytes and human fibroblasts. In

addition, it could induce IL-2 synthesis by HSB2 T-cell clones. It is noteworthy that when the activity of both monocyte and 3B6 IL-1 were standardized using the thymocyte assay, the specific activity of 3B6 IL-1 appeared to superimpose on that of monocyte IL-1 in both assays. Its 13.5-kDa size and its acidic pI also suggest some similarity with monocytic IL-1. However, the purification to homogeneity of this IL-1 material allowed us to demonstrate crucial differences. In contrast to monocyte IL-1, it could not be inhibited by an anti-IL-1 serum raised against monocyte-derived IL-1, which inhibits both IL-1α and IL-1β activities (data not shown) (Fig. 6). Also, 3B6 IL-1 is devoid of pyrogenic activity (17). Finally, as detailed elsewhere (17), its N-terminal amino acid sequence was found to be: Val-Lys-Gln-Ile-Glu-Ser-Lys-Thr-Ala-Phe-Gln-Glu-Ala-Leu-Asp, which offers no similarity with any of the sequences reported for either human IL-1α or -β. The IL-1 produced by the 3B6 line thus appeared to represent a newly described type of IL-1.

An intriguing possibility could be that some similarities exist between this 3B6 IL-1 and a T-lymphocyte-derived factor reported by Yodoi and co-workers (30) and named (ATL) adult T-cell leukemia-derived factor (ADF) (30). ADF is produced by a human T-cell leukemia virus type 1-infected T-cell line and was described as a potent inducer of IL-2 receptor expression on the "natural killer" cell line YT (31). ADF was shown to be a 15- to 20-kDa protein, with acidic pI value below 5 (30). ADF actually exhibited very efficient IL-1 activity whereas its N-terminal amino acid sequence differed from both IL-1α and -β (32). Interestingly, preliminary results show that 3B6 IL-1 could also very efficiently induce IL-2 receptor expression on the YT line. Therefore, ADF and 3B6 IL-1 could represent "lymphocytic" IL-1 species distinct from the monocytic forms of IL-1.

Finally, we present data suggesting that the 3B6 clone could use IL-1 as an autocrine growth factor. Optimal proliferation of 3B6 cells appeared to be dependent upon the presence of growth factors as provided by FCS and upon the cell concentration. Both these requirements could, however, be bypassed by the addition to the culture medium of purified IL-1, either monocytic or derived from the 3B6 line itself. These data suggest that IL-1 may contribute significantly to the proliferation of 3B6 cells. The role of autocrine growth factor in the EBV transformation of B cells has been suggested by Gordon *et al.* (33), who found a 25-kDa molecule with B-cell growth factor activity in the conditioned medium from EBV-containing LCL cells. It is noteworthy that spontaneously, the supernatants of 3B6 also contained B-cell growth factor activity (assessed by the induction of proliferation of anti-μ-stimulated normal B cells) that could be separated from IL-1 during the process of biochemical purification (data not shown). It is thus tempting to speculate that in the 3B6 clone, as well as in other LCL cells, IL-1 could promote the autocrine growth of B cells, possibly in synergy with B-cell growth factor. An interesting hypothesis could be that IL-1-negative subclones (e.g., 2E5 cells) indeed constitutively produce other factors, such as B-cell growth factor, which complement the lack of IL-1. The role of IL-1 production in the process of B-cell transformation by the EBV remains to be investigated.

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1. Smith, K. A., Lachman, L. B., Oppenheim, J. J. & Favata, M. F. (1980) *J. Exp. Med.* **151,** 1551–1556.
2. Oppenheim, J. J., Mizel, S. B. & Meltzer, M. S. (1979) in *Biology of the Lymphokines*, eds. Cohen, S., Pick, E. & Oppenheim, J. J. (Academic, New York), pp. 291–323.
3. Gery, I. & Waksman, B. H. (1972) *J. Exp. Med.* **136,** 143–155.
4. Dinarello, C. A. (1984) *Rev. Infect. Dis.* **6,** 51–95.
5. Schmidt, J. A., Mizel, S. B., Cohen, D. & Green, I. (1982) *J. Immunol.* **128,** 2177–2182.
6. Dinarello, C. A. (1985) *J. Clin. Immunol.* **5,** 287–297.
7. Auron, P. E., Webb, A. C., Rosenwasser, L. J., Mucci, S. F., Rich, A., Wolff, S. M. & Dinarello, C. A. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7907–7911.
8. March, C. J., Mosley, B., Larsen, A., Cerretti, D. P., Braedt, G., Price, V., Gillis, S., Henney, C. S., Kronheim, S. R., Grabstein, K., Conlon, P. J., Hopp, T. P. & Cosman, D. (1985) *Nature (London)* **315,** 641–647.
9. Cameron, P., Limjuco, G., Rodkey, J., Bennett, C. & Schmidt, J. A. (1985) *J. Exp. Med.* **162,** 790–801.
10. Van Damme, J., De Lay, M., Opdenakker, G., Billiau, A., De Somer, P. & Van Beeumen, J. (1985) *Nature (London)* **314,** 266–268.
11. Lomedico, P. T., Gubler, U., Hellmann, C. P., Dukovich, M., Giri, J. G., Pan, Y.-C. E., Collier, K., Semionow, R., Chua, A. O. & Mizel, S. B. (1984) *Nature (London)* **312,** 458–462.
12. Furutani, Y., Notake, M., Yamayoshi, M., Yamagishi, J., Nomura, H., Ohue, M., Furuta, R., Fukui, T., Yamada, M. & Nakamura, S. (1985) *Nucleic Acids Res.* **13,** 5869–5882.
13. Luger, T. A., Stadler, B. M., Katz, S. I. & Oppenheim, J. J. (1981) *J. Immunol.* **127,** 1493–1498.
14. Fontana, A., Hengartner, H., Tribolet, N. & Weber, E. (1984) *J. Immunol.* **132,** 1837–1844.
15. Wahl, S. M., Malone, D. G. & Wilder, R. L. (1985) *J. Exp. Med.* **161,** 210–222.
16. Matsushima, K., Procopio, A., Abe, H., Scala, G., Ortaldo, J. R. & Oppenheim, J. J. (1985) *J. Immunol.* **135,** 1132–1136.
17. Rimsky, L., Wakasugi, H., Ferrara, P., Robin, P., Capdevielle, J., Tursz, T., Fradelizi, D. & Bertoglio, J. (1986) *J. Immunol.* **136,** 3304–3310.
18. Whitehead, A. S., Colten, H. R., Chang, C. C. & DeMars, R. (1985) *J. Immunol.* **134,** 641–643.
19. Dinarello, C. A., Renfer, L. & Wolff, S. M. (1977) *J. Clin. Invest.* **60,** 465–472.
20. Mizel, S. B., Oppenheim, J. J. & Rosenstreich, D. L. (1978) *J. Immunol.* **120,** 1497–1503.
21. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. (1978) *J. Immunol.* **120,** 2027–2032.
22. Kasahara, T., Mukaida, N., Hatake, K., Motoyoshi, K., Kawai, T. & Nakano, K. S. (1985) *J. Immunol.* **134,** 1682–1689.
23. Gillis, S., Ferm, M. M., Ou, W. & Smith, K. A. (1978) *J. Immunol.* **120,** 2027–2032.
24. Wakasugi, H., Harel, A., Dokhelar, M. C., Fradelizi, D. & Tursz, T. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6028–6031.
25. Wakasugi, H., Harel, A., Dokhelar, M. C., Fradelizi, D. & Tursz, T. (1984) *J. Immunol.* **132,** 2939–2947.
26. Wakasugi, H., Dokhelar, M. C., Garson, D., Bellan, A. H., Fradelizi, D. & Tursz, T. (1985) *Eur. J. Immunol.* **15,** 256–261.
27. Oppenheim, J. J., Scala, G., Kuang, Y. & Matsushima, K. (1983) *Prog. Immunol.* **5,** 285–293.
28. Scala, G., Kuang, Y. D., Hall, R. E., Muchmore, A. V. & Oppenheim, J. J. (1984) *J. Exp. Med.* **159,** 1637–1652.
29. Matsushima, K., Tosato, G., Benjamin, D. & Oppenheim, J. J. (1985) *Cell. Immunol.* **94,** 418–426.
30. Teshigawara, K., Maeda, M., Nishino, K., Nikaido, T., Uchiyama, T., Tsudo, M., Wano, Y. & Yodoi, J. (1985) *J. Mol. Cell. Immunol.* **2,** 17–26.
31. Yodoi, J., Teshigawara, K., Nikaido, T., Fukui, K., Noma, T., Honjo, T., Takigawa, M., Sasaki, M., Minato, N., Tsudo, M., Uchiyama, T. & Maeda, M. (1985) *J. Immunol.* **134,** 1623–1630.
32. Yodoi, J., Tagaya, Y. & Maeda, M. (1987) in *Lymphokines*, ed. Smith, K. (Academic, New York), in press.
33. Gordon, J., Ley, S. C., Melamed, M. D., English, L. S. & Hughes-Jones, N. C. (1984) *Nature (London)* **310,** 145–147.