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IN VITRO PRODUCTION OF INTERLEUKIN 1 BY NORMAL AND MALIGNANT HUMAN B LYMPHOCYTES[1]

VITO PISTOIA,*† FEDERICO COZZOLINO,*‡ ANNA RUBARTELLI,* MARIA TORCIA,‡ SILVIO RONCELLA,* AND MANLIO FERRARINI*†[2]

*From the *Servizio di Immunologia Clinica, Istituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV, 10 - 16132 Genova; and †Istituto di Oncologia Clinica e Sperimentale, Università di Genova, Viale Benedetto XV, 10 - 16132 Genova; and ‡Istituto di Clinica Medica II, Università di Firenze, Viale Pieraccini, 18 - 50139 Firenze*

In this study, the capacity of normal and neoplastic B lymphocytes to release interleukin 1 (IL 1) has been investigated. Peripheral blood B cells from normal donors were isolated by depletion of E rosetting cells and by positive selection of cells expressing surface immunoglobulin (sIg) or the B1 marker. Peripheral blood B cells from patients with B cell chronic lymphocytic leukemia (B-CLL) were purified by removal of E rosetting cells followed by complement-mediated cytotoxicity with selected monoclonal antibodies. All of the normal B cell suspensions and the large majority of the B-CLL cells produced in culture high amounts of IL 1 in the absence of any apparent stimulus. Control experiments ruled out that small numbers of monocytes in the B cell suspensions could represent the source of IL 1. These data support the contention that B cells participate to the immune response as accessory cells for T cell activation not only by physically presenting antigen, but also by releasing IL 1.

In recent years, numerous studies have shown that lymphocytes produce soluble mediators generally called lymphokines (1–5). Some of these, for example interleukin 2 (IL 2), deliver signals to lymphocytes and sustain their proliferation (6). Others, such as interferon-γ, activate and potentiate effector mechanisms of immunity (7). Subpopulation studies have demonstrated that both T cells (1–6) and natural killer (NK) cells (8–14) produce lymphokines whereas little is known about the capacity of B cells to release these factors. Lack of information in this regard may be attributed to the widespread concept that the sole B cell function is that of producing specific antibodies. Consequently, most studies have been addressed to the process of B cell maturation, both at the cellular (15) and at the molecular (16) level. More recently, however, it has been shown that B lymphocytes can function as antigen-presenting cells for T lymphocytes (17–25) and consequently subserve an accessory role for the induction of immune response.

In this study, we have investigated whether or not B cells release interleukin 1 (IL 1), a cytokine that appears to play an important role in the early steps of T cell activation and in the control of the inflammatory response (as reviewed in Reference 26). We demonstrate that peripheral blood B cells from normal individuals and from patients with B cell chronic lymphocytic leukemia (B-CLL)[3] are effective producers of IL 1.

MATERIALS AND METHODS

Cell fractionation procedures. Peripheral blood mononuclear cells (MNC) from normal donors were separated on Ficoll-Hypaque (F-H) density gradients. Monocytes were partially removed by adherence. T cells were removed from the suspensions by rosetting with neuraminidase-treated sheep erythrocytes (9). Cells expressing surface immunoglobulin (sIg) were isolated by rosetting non-T cells with ox erythrocyte coated with rabbit $F(ab')_2$ anti-human Ig serum (27). Cells expressing the B1 marker were purified by an indirect rosetting technique. Briefly, non-T cell fractions were treated with OKM1 monoclonal antibody (Ortho Diagnostic Systems, Milano, Italy) and complement as reported (13). These non-T cells (3×10^7) were treated with 100 μl of the B1 monoclonal (Coulter-Kontron, Milano, Italy) and were rosetted with 2 ml of 5% ox erythrocytes coated with a purified rabbit anti-mouse Ig antibody (27). After a 30-min incubation at 4°C, rosetting cells ($B1^+$ cells) were separated from nonrosetting cells ($B1^-$ cells) on F-H density gradients. In some experiments, monocytes were recovered from plastic after adherence by a rubber policeman.

Cells from 12 B-CLL patients were also studied. The diagnosis of B-CLL was established by clinic, morphologic, and immunologic criteria. All of the patients had 25,000 white blood cells/mm^3 or more (90% lymphocytes). B-CLL MNC suspensions were depleted of E rosettes as above and treated with the OKM1 and Leu-7 monoclonal antibodies and complement (13). In some experiments, B-CLL B cells were purified as $B1^+$ cells by using the technique described above.

Cell surface marker analysis. Cells with receptors for sheep erythrocytes or sIg were detected as reported (13). The murine monoclonal antibodies used in this study were the pan-T reagent OKT3 (Ortho Diagnostic System); the B cell-specific antibody B1; the large granular lymphocytes (LGL)-specific reagents Leu-7 and Leu-11b (Becton Dickinson Laboratory System, Milano, Italy); the anti-monocyte-macrophage monoclonal, which also stains LGL, OKM1; and the anti-HLA-DR monoclonal PTF 29/12 (28) that was kindly donated by Dr. G. Damiani. All of the above reagents were used in indirect immunofluorescence with a fluoroscein isothiocyanate (FITC)-conjugated rabbit $F(ab')_2$ anti-mouse Ig. Preparations were observed with a Leitz Orthoplan fluorescence microscope. The percent of positive cells was calculated on at least 200 cells per preparation. In some experiments, cell suspensions were also stained for the cytochemical localization of α-naphthyl acetate esterase (ANAE) as reported (9).

Preparation of culture supernatants. $B1^+$ cells or recovered adherent cells were cultured (1×10^6/ml) for 48 hr in RPMI 1640 supplemented with 10% fetal calf serum (FCS) (Flow Laboratories, Milano, Italy) in 24 macro-well plates (Flow). Supernatants were harvested after centrifugation and were stored at −20°C.

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[1] This work was supported in part by grants from the Italian Consiglio Nazionale delle Ricerche (P. F. Oncologia) and from the Ministero della Pubblica Istruzione.
[2] Address reprint requests to Vito Pistoia, M.D., Servizio di Immunologia Clinica, Istituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV, 10 16132 Genova.

[3] Abbreviations used in this paper: sIg, surface immunoglobulin; B-CLL, B cell chronic lymphocytic leukemia; MNC, mononuclear cells.

IL 1 assay. IL 1 activity was assayed by the conventional thymocyte assay (29). In brief, thymocytes from C3H/HeJ mice were cultured for 72 hr in 96-well flat bottomed plates (Flow) at the concentration of 1.5×10^6/well in the presence of phytohemagglutinin (PHA; Difco, Detroit, MI) (1 μl/ml) alone, or of PHA plus serial dilutions of the supernatant to be tested. At the end of this time, cells were pulsed with 1 μCi of [^{3}H]thymidine for 8 hr, were harvested, and were counted. One unit of IL 1 was defined as the amount of supernatant capable of doubling the [^{3}H]thymidine uptake by 1.5×10^6 PHA-stimulated thymocytes.

Because the presence of IL 2 in test supernatant could interfere with the results of the IL 1 assay, IL 2 activity was measured in all of the conditioned media as reported (14). Briefly, the capacity of the following cells to be stimulated was determined: a PHA-induced normal T cell line that had been expanded and kept in culture for 2 mo by adding exogenous IL 2; this line was unresponsive to PHA in the absence of any irradiated accessory cell; and the murine cell line CTLL-2. Both assays consistently failed to detect any IL 2 activity in B cell or monocyte supernatants.

Molecular weight determination of IL 1. To 80 ml of normal B cell or monocyte culture supernatants $(NH_4)_2SO_4$ was added to a final concentration of 75%. The precipitate was centrifuged, was resuspended in phosphate-buffered saline containing 50 μg/ml gentamicin (PBS), and was dialyzed extensively against the same buffer. Aliquots of 3 ml were then gel filtered on a Sephadex G-75 Superfine (Pharmacia) column equilibrated with PBS and were calibrated with the following markers: aldolase (158,000 m.w.), human serum albumin (69,000 m.w.), isolated Ig light chains (23,000 m.w.), and cytochrome c (12,300 m.w.). A flow rate of 10 ml/hr was used, and 2-ml fractions were collected. Aliquots of the fractions were 0.22 μm filtered and were assayed for IL 1 activity as described above.

RESULTS

In initial studies, normal B cells were isolated from the non-T cell fraction of peripheral blood by rosetting with ox erythrocytes coated with anti-Ig antibodies (Table I). sIg$^+$ cells produced large amounts of IL 1 (Table II), but monocyte contamination of these cell populations was too high to indicate conclusively that B cells could be the source for the detectable cytokine (Table I).

To overcome these difficulties, the following purification procedure was used: peripheral blood non-T cells partially depleted of monocytes by adherence to plastic were treated with OKM1 antibody and complement. The remaining cells were subsequently fractionated into B1$^+$ and B1$^-$ cells by an indirect rosetting technique. The B1$^+$ cell suspensions contained 95% or more B cells (as detected by staining for sIg), thus being considerably less contaminated than the sIg$^+$ cell preparations (Table I). The reasons for the improved B cell purification are depletion of OKM1$^+$ monocytes by complement-mediated cytotoxicity and failure of the B1 monoclonal antibody to bind to monocytes, as opposed to the anti-Ig reagent, which consistently reacted with sIg molecules passively absorbed on a proportion of monocytes. The capacity of B1$^+$ cells to produce IL 1 was tested and was compared with that of autologous monocytes recovered after adherence to plastic surfaces (Table II). These adherent cells contained 90% ± 5% monocytes, as determined by reactivity with the OKM1 monoclonal antibody and by the characteristic pattern of ANAE staining. Minor contaminants were represented by B cells (4%), T cells (2%), and LGL (2%). B1$^+$ cells released IL 1 in quantities that, on a per cell basis, were comparable with, or higher than, those produced by monocytes (Table II). This finding makes it unlikely that the few monocytes present in the B1$^+$ cell suspensions were responsible for IL 1 production. The possibility that B cells released IL 1 as a consequence of endotoxin stimulation was ruled out by the observation that the batch of FCS used had a very low endotoxin content, as assessed by the Limulus assay. Furthermore, B cells cultured in medium containing 1% human serum albumin in the place of FCS retained the ability to produce IL 1.

B cells from 12 B-CLL patients were also tested for IL 1 release (Table III). B cells were isolated from peripheral blood MNC by removing E rosettes and subsequently treating the suspensions with the OKM1 and Leu-7 monoclonal antibodies and complement. The last treatment was necessary, because these suspensions still contained 4% ± 1 OKM1$^+$ and 3% ± 1 Leu-7$^+$ cells. After this procedure, the suspensions were enriched for B cells up to 95% or greater (Table I). In some cases, B cells were purified by enrichment for B1$^+$ cells by using rosette methods, and results were virtually identical. Nine of the 12 cases studied (75%) produced IL 1 in amounts that

TABLE I

Surface phenotype of normal and B-CLL-enriched B cell suspensions

Surface Marker	Percent of Positive Cells[a]		
	sIg$^+$ cells[b]	B1$^+$ cells[c]	B-CLL B cells[d]
sIg	92 ± 5[e]	95 ± 4	N.D.[f]
B1	80 ± 6	96 ± 3[e]	95 ± 3
HLA-DR	95 ± 3	98 ± 2	98 ± 5
OKT3	<1	<1	<1
OKM1	15 ± 3	<2	<1
Leu-11	4 ± 1	<1	<2
Leu-7	2 ± 0.3	<1	<1

[a] Mean ± SE from three different experiments. The SE is calculated as the square root of the pooled variance divided by the overall number of samples. The homogeneity of variance between experiments was demonstrated, and no significant difference between the means was documented.

[b] B cells were isolated by direct rosetting according to the expression of sIg (see *Materials and Methods*).

[c] B cells were isolated by indirect rosetting according to the expression of B1 antigen (see *Materials and Methods*).

[d] B-CLL B cells were obtained by depletion of E rosetting cells followed by treatment with OKM1 and Leu-7 monoclonal antibodies plus complement.

[e] Detected by rosetting during the separation procedures.

[f] Not done because of the low-density expression of sIg by B-CLL B cells.

TABLE II

IL 1 production by cell suspensions enriched for B cells and monocytes

Supernatants from	IL 1 Activity[a] (U/ml)		
	Expt. 1	Expt. 2	Expt. 3
sIg$^+$ cells	130	116	110
B1$^+$ cells	129	118	98
Autologous monocytes[b]	78	84	79

[a] Supernatants were tested at different dilutions, and the reported values are relative to those yielding the highest activity. IL 1 produced by 1×10^6 cells cultured for 48 hr.

[b] Monocytes were recovered from plastic after adherence.

TABLE III

IL 1 production by B cells from B-CLL patients

Patient	IL 1[a] (U/ml)
A	160
B	0
C	0
D	135
E	42
F	0
G	93
H	52
I	82
L	32
M	21
N	12

[a] Supernatants were tested at different dilutions, and the reported values are relative to those yielding the highest activity. IL 1 produced by 1×10^6 cells cultured for 48 hr.

were comparable with those of normal B cells (Table III). Interestingly, neither normal nor malignant B cells released IL 2, measured by the support of IL 2-dependent T cell growth. Thus, the IL 1 activity detected by the thymocyte assay could not be due to the presence of IL 2 (data not shown). Normal B cell supernatants were ammonium sulphate precipitated and were subsequently gel filtered over a Sephadex G75 column. Figure 1 shows the elution pattern of the B cell-derived factor with IL 1 activity, which is superimposable to that released by normal monocytes. As evident, the apparent m.w. of both molecules is in the range between 15,000 and 20,000.

DISCUSSION

Our study demonstrates that peripheral blood B cells from both normal donors and B-CLL patients produce IL 1. Normal and malignant B cells apparently released IL 1 constitutively in the absence of any stimulation. On the basis of m.w. determination (15,000 to 20,000), the available data suggest that this factor could be similar to that released by monocytes. The possibility exists that cells were induced to release IL 1 by contact with FCS or even plastic tissue culture ware. Alternatively, IL 1 may have been produced by B cells preactivated in vivo (30). While this manuscript was in preparation, Matsushima et al. reported similar data (31).

The results reported here were obtained by using monoclonal antibodies and rosetting techniques for cell fractionation. Normal B cells were isolated from peripheral blood non-T cells according to the expression of sIg or of the B1 marker. B cell enrichment within the $B1^+$ fraction proved by far superior to that detected in sIg^+ cells, which contained relevant proportions of monocytes. In addition, B cell suspensions could be additionally purified by treatment with anti-monocyte monoclonal antibodies and complement. In theory, it cannot be excluded that the separation procedures used in these experiments select a small subset of slightly adherent, $OKM1^-$, Fc receptor positive cells, which are highly efficient IL 1 producers and co-purify with B cells through nonspecific binding of the B1 monoclonal antibody or of antibody coated ox erythrocytes. Furthermore, Fc receptor interaction with ox erythrocytes could deliver an effective signal for cytokine release. However, it is unlikely that IL 1 production was attributable to contaminant monocytes for the following reasons: the high degree of purity for the normal B cell fractions used; the finding that on a per cell basis, B cell-enriched suspensions were as efficient IL 1 producers as autologous monocytes, and the observation that CLL B cells purified by negative selection methods (i.e., removal of E rosetting cells followed by killing of residual non-B cells with monoclonal antibodies and complement) produced IL 1 in amounts comparable with those of normal B cell suspensions. Nonetheless, because a small contaminant of monocytes was still present in the B cell suspensions, the possibility that monocytes cooperate with B cells in inducing IL 1 release by the latter cells cannot be dismissed.

We have not yet observed a relationship between IL 1 production and other characteristics of the single B-CLL cases, such as lymphocyte counts, treatment, duration, or stage of disease. Previously we have shown that individual B-CLL clones reach different maturational stages in vivo, which can be assessed on the basis of the capacity of the cells to produce and secrete Ig molecules and of their responsiveness to growth and differentiation factors (32, 33). When these parameters were used to determine the maturational stages of the B-CLL cases studied, no correlation was found between the degree of maturation of the malignant B cells and IL 1 production. Whether or not B-CLL cells that release IL 1 in vitro also produce the same cytokine in vivo is presently unknown; addi-

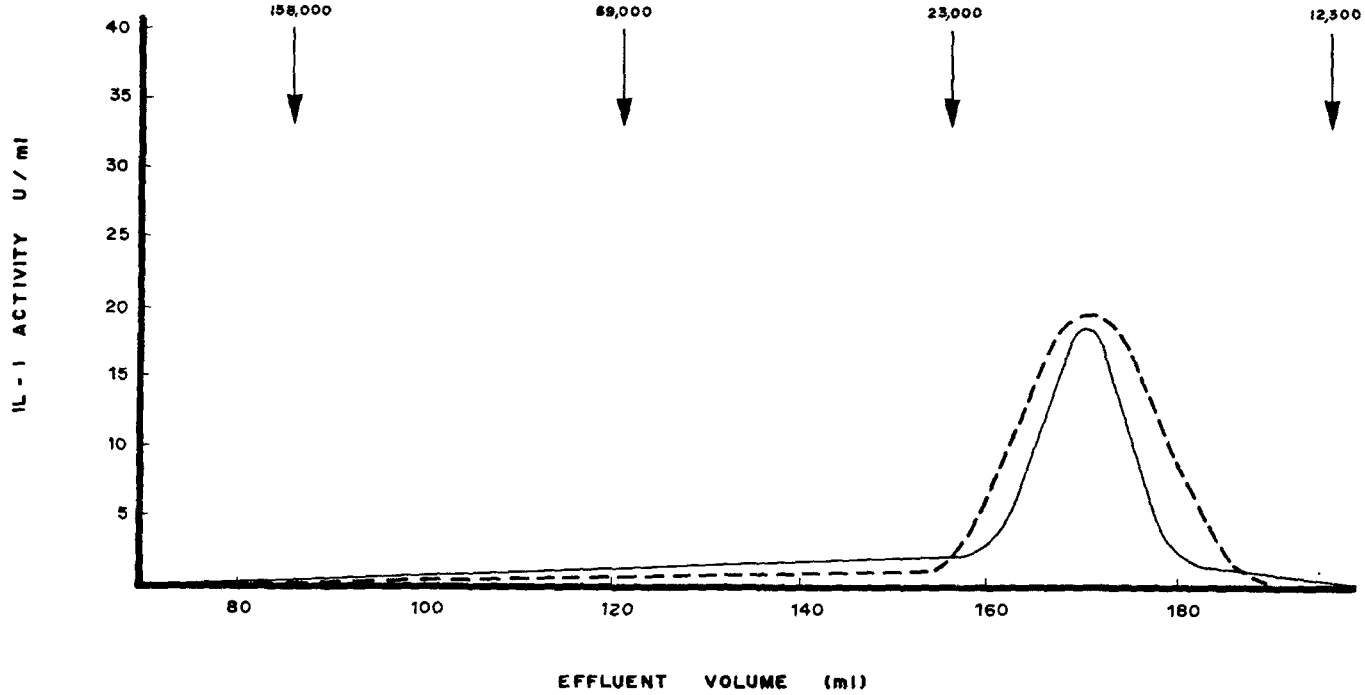

Figure 1. Elution patterns of normal B cell-derived (—) or normal monocyte-derived (— — —) factors with IL 1 activity. Culture supernatants were ammonium sulphate precipitated, and precipitates were applied to a Sephadex G-75 column; fractions were collected, and aliquots of each fraction were assayed for IL 1 activity.

tional studies on serum and urine (34) from these patients are now in progress.

Several explanations for the heterogeneity in IL 1 production by B-CLL cells can be considered. First, IL 1 production could be restricted to a B cell subset of which the lymphokine producing B-CLL cases represent the counterpart. Second, IL 1 may be released at a particular B cell maturational stage that cannot yet be defined with the available methods. Finally, all B-CLL cells could produce IL 1, but in some cases, this lymphokine could be absorbed on the malignant cells. This possibility is suggested by the following observations: IL 1 can affect both proliferation and differentiation of B cells (35–40), and some Epstein Barr virus-infected B cell lines produce IL 1, which can, at least in part, maintain their proliferative capacity (41).

The demonstration that B cells release IL 1 is in accordance with their capacity to present antigens (17–25) and reinforces the view that B cells function as accessory cells for T cell proliferation. Finally, in view of the role that IL 1 plays for the induction and/or maintainance of inflammatory reaction (26), our data suggest a novel B cell function in this particular feature of the response to pathogens. In this connection, it is of note that we have preliminarly shown that both normal and malignant B cells are also capable of producing considerable amounts of granulocyte-macrophage colony stimulating factor (Pistoia et al., manuscript in preparation).

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