Brief Definitive Report

INTERLEUKIN 2–INDUCED PROLIFERATION OF LEUKEMIC HUMAN B CELLS

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In the majority of chronic lymphocytic leukemias (CLL), proliferative B lymphocytes are thought to be frozen in vivo at a given maturation stage (1). Recently, several studies have emphasized that CLL cells could be driven to differentiate into plasma cells in vitro (with or without proliferation) upon triggering by mitogens (2, 3, 4) or T cell–derived factors (5, 6). Thus far, however, no study has been designed to elucidate whether leukemic B cells can be triggered by T cell–derived lymphokines that are known to induce normal B cells to proliferate. In preliminary experiments, we found that leukemic B cells from six of nine CLL patients, when stimulated with anti-μ antibody and/or with *Staphylococcus aureus* Cowan strain I (SAC), proliferate in the presence of various T cell–derived conditioned media, which potentially contained a number of lymphokines (unpublished data). Absorption experiments suggested that interleukin 2 (IL-2) was a potent growth factor for these leukemic B cells. We report here that purified recombinant IL-2 is indeed able to induce leukemic cells from some patients to proliferate. These data, obtained with monoclonal populations of B cells, which are likely free of contaminating T cells, provide further evidence that IL-2 can trigger activated B cells to proliferate.

Material and Methods

Isolation and Characterization of Lymphoid Cells. Nine cases of well-documented B cell CLL were studied. Mononuclear cells were purified from blood by centrifugation on a Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient. Although the percentage of E-rosetting cells was very low ($\leq$1% except in patient ROS) B cells were purified by one or two cycles of depletion by Ficoll-Hypaque centrifugation of those cells forming E rosettes with 2 aminoethylisothiouronium bromide–treated sheep red blood cells. Cell marker studies showed that >99% of the cells were monoclonal B cells bearing surface IgM and IgD in eight cases, and IgG in one.

Normal B cells were purified from the spleen of a patient with idiopathic thrombocytopenic purpura. This B cell population contained <1% T cells and >95% surface Ig–positive cells.

Monoclonal antibodies (mAb) against B cells (against B1 antigens), IL-2 receptor (TAC), and E-rosette receptor (D66 and OKT11) were kind gifts of Dr. S. F. Schlossman and L. E. Nadler (Dana-Farber Cancer Institute, Boston, MA), T. A. Waldmann (National

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Institutes of Health, Bethesda, MD), and L. Boumsell (INSERM U93). OKT3 was obtained from Ortho Pharmaceutical, Raritan, NJ.

Sources of IL-2. Semipurified IL-2 was prepared from phytohemagglutinin-stimulated peripheral blood mononuclear cell supernatants, precipitated by ammonium sulfate, and recovered after gel filtration on ultrogel AcA 54 (Industrie Biologique de France, Villeneuve la Garenne, France). Purified recombinant IL-2 (with an estimated specific activity of 10^7 U/mg protein) was obtained from Biogen S.A. (Geneva, Switzerland). This preparation contained 97.3% recombinant IL-2.

Microassay for IL-2 activity determination was performed on the IL-2-dependent murine cell line, CTLL, according to Gillis et al. (7). We define one unit of IL-2 as the activity contained in a sample dilution yielding a proliferation equal to 50% of the maximum [^{3}H]thymidine uptake (in cpm) of the first French Workshop standard IL-2 preparation (8). In these conditions, semipurified and recombinant IL-2 had an activity of 14 and 1,900 U/ml, respectively. Therefore, 1 U corresponds to 10.5 ng recombinant IL-2. One U IL-2 contained <0.05 pg endotoxin, as assessed by the limulus amebocyte assay.

Assay of B Cell Proliferation. Proliferation was determined after preactivation of B cell by SAC or anti-μ antibody. In the SAC assay (9), cells were cultivated at a concentration of 10^6/ml for 3 d in 250-ml flasks (1-53373, Nunc, Roskilde, Denmark), with or without SAC (1:10,000 vol/vol). SAC was prepared in our laboratory (killed by incubation in 1.5% formaldehyde, followed by heat treatment). This preparation has no mitogenic activity on purified T cells. At day 3 (unless otherwise specified) cells were washed three times in complete medium, and distributed at a cell concentration of 10^5 or 5×10^4 cells/well in 200 μl medium, in 96-well, flat-bottom microtiter plates (67008, Nunc) with serial dilutions of purified recombinant IL-2 for three additional days.

In the costimulatory assay with anti-μ antibody (10), a mouse IgG1 mAb against human μ chains (prepared in our laboratory) was coupled to Sepharose CL 4 B (2 mg/ml) (Pharmacia Fine Chemicals) and used at a concentration of 1 μg/ml; this dose was selected on the basis of preliminary experiments on normal and leukemic B cells. 10^5 leukemic, or 5×10^4 normal B cells were incubated in 200 μl medium in microtiter plates, in the presence or absence of anti-μ antibody, with serial dilutions of IL-2 to be tested, for 3 or 6 d.

In both assays, 1 μCi/well [^{3}H]thymidine ([^{3}H]TdR) (Commisariat pour l'Energie Atomique, Gif-sur-Yvette, France) was added during the last 18 h of culture. Cells were thereafter harvested on glass fiber filters, and incorporation of [^{3}H]TdR was measured by standard liquid scintillation counting.

Results and Discussion

The main result of this study is the demonstration that purified recombinant IL-2 is able to trigger leukemic B cells from six of nine CLL cases to proliferate after preactivation. The effect of recombinant IL-2 on CLL B cells was assessed in two different B cell proliferative assays, i.e. after activation by SAC or anti-μ. IL-2 at various concentrations (0.01–3 U/ml) was added at day 0 in the costimulatory assay with anti-μ, or at day 3 after preactivation by SAC. In the SAC assay, cells from three of nine patients proliferated in response to SAC alone: in two cases (ETA and ROS) the response was weak, with a peak at day 3; in the third case (BAU), the proliferative response was high, with a peak at day 6. Cells that responded to SAC (from the aforementioned three patients), proliferated in response to IL-2 (Fig. 1). No response was observed among cells from the other six patients.

Costimulation of purified leukemic B cells with IL-2 and anti-μ gave only a marginal response (<1,500 cpm) at day 3. When the incubation time was extended to 6 d, a clearcut pattern of IL-2 reactivity emerged in the leukemic

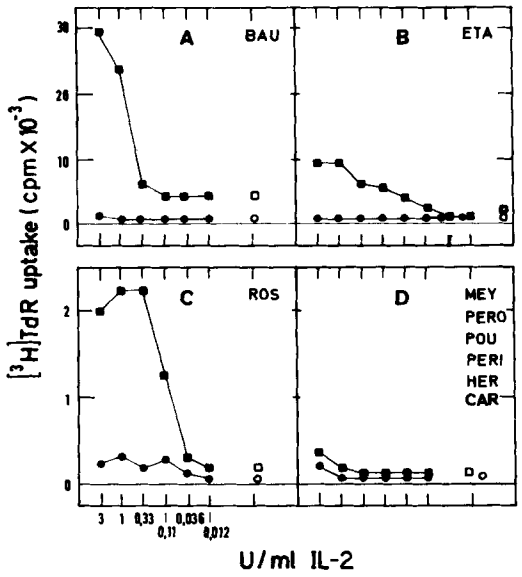

FIGURE 1. Proliferation of SAC-activated CLL B cells in response to recombinant IL-2: 10^6 cells/ml were incubated for 3 d (*B*, *C*, and *D*) or 9 d (*A*) with (■) or without (●) SAC (1:10,000 vol/vol), washed three times, and resuspended at 10^5 (*A*, *C*, and *D*) or 5×10^4 cells/well (*B*) with serial dilutions of recombinant IL-2. 1 μCi of [^{3}H]TdR was added during the last 18 h of the three additional days of culture. The proliferation of cells in the absence of IL-2, with or without preactivation by SAC, is shown by the corresponding open symbols (□, ○).

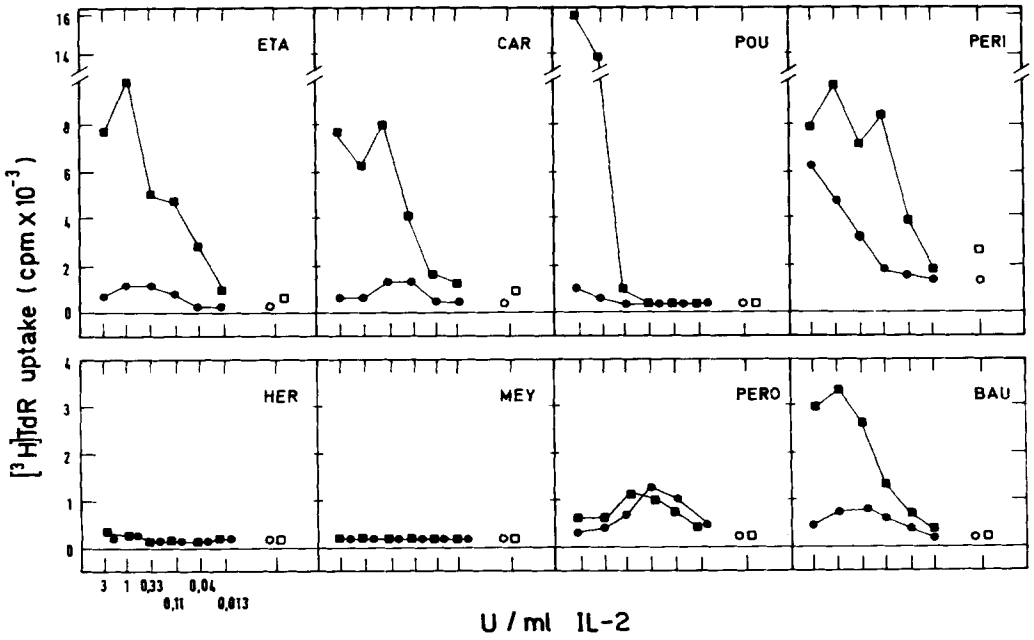

FIGURE 2. Proliferative response of anti-μ–activated CLL B cells to recombinant IL-2: 10^5 cells/well were incubated for 6 d with (■) or without (●) insolubilized anti-μ mAb (1 μg/ml), and with 0.01–3 U/ml recombinant IL-2. The proliferation of cells in the absence of recombinant IL-2, with or without anti-μ, is shown by the corresponding open symbols (□, ○).

cells (Fig. 2). Interestingly, cells from one patient (PERI) proliferated to IL-2 alone, the response being enhanced after preactivation with anti-μ. The cells from four other patients (ETA, CAR, POU, and BAU) proliferated in response to IL-2 when simultaneously activated by anti-μ. Finally, no response was noted in three cases (HER, MEY, and PERO). Cells from patient ROS, which featured surface IgG and which proliferated in response to IL-2 after SAC stimulation were not studied in this assay. Cells from a single patient proliferated slightly in the presence of anti-μ alone; in the other cases, cells were preactivated without detectable proliferation, as actually occurs for normal B cells triggered by low doses of anti-μ (11). Of note, the CLL cell response to IL-2 peaked at day 6, i.e. later than the response of anti-μ–activated normal B cells to growth factors (11).

The amount of IL-2 needed for the leukemic cells to proliferate varied from patient to patient. In one case (POU), the cells were responsive only at high concentrations of IL-2 (3 and 1 U/ml), whereas in the other four cases (ETA, BAU, PERI, and CAR), the dose/response curves were similar, with a peak of ~1 U/ml, and background-level proliferation at a concentration of 0.02 U/ml. With regard to these results, it is noteworthy that our IL-2 assay on the murine IL-2-dependent CTLL line is able to detect ~0.01 U/ml IL-2.

The use of leukemic B cells makes it very unlikely that the observed effect was due to residual T or accessory cells. After one or two cycles of E-rosette depletion, the leukemic population tested was almost pure, i.e. contained >99% surface Ig–positive monoclonal cells and <0.02% T cells, and <0.01% polyclonal B cells, as estimated by cell markers at day 0, 3, and 6 of culture. On the other hand, to exclude the possibility that the responsiveness to IL-2 was a unique feature of these leukemic B cells, we performed similar experiments on spleen-enriched B cell populations. Again, recombinant or semipurified IL-2 induced activated B cells to proliferate in a dose-dependent fashion (Fig. 3). These results are in

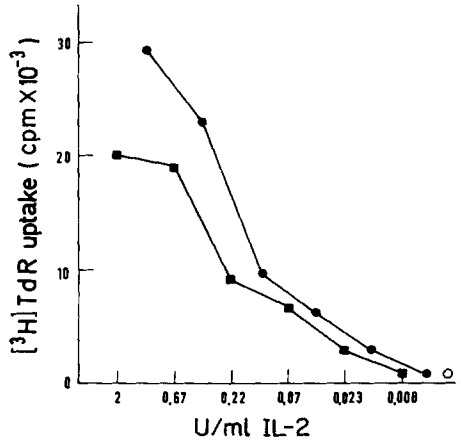

FIGURE 3. Proliferative response of SAC-preactivated normal B cells from spleen to IL-2. 10^6 cells/ml purified B cells were cultivated for 3 d with SAC (1:10,000 vol/vol), washed, and recultivated at 10^5 cells/well for three additional days with 0.01–2 U/ml of IL-2, either semipurified (●) (see Materials and Methods), or recombinant (■). Proliferation with medium alone is shown by the open symbol (○). In all cases, standard deviation was <10% of the response.

agreement with recently published data. IL-2 receptors have been found on ~25% of anti-μ- or SAC-activated normal B cells (12, and our unpublished results). Herein, the study of IL-2 receptor expression by leukemic cells was hampered, in some cases, by nonspecific binding to a strong Fc receptor. However, fresh cells from two of six patients were weakly stained by anti-TAC; in one case, they proliferated in response to IL-2 without preactivation (patient PERI), and in the other case (BAU), a second signal, delivered either by SAC or anti-μ, was needed for proliferation to occur. After activation by SAC or anti-μ, the cells from two other patients, although responsive to IL-2, did not exhibit detectable amounts of TAC antigen, possibly because of a low density of receptor. Finally, in one case studied, anti-TAC was able to inhibit recombinant IL-2–induced proliferation of leukemic cells up to a 10^{-5} dilution, providing further evidence that IL-2 is indeed the active lymphokine in our assay.

Altogether, our findings demonstrate that B cells from a majority of CLL, as well as normal B cells, are susceptible to IL-2. The role of IL-2 in B cell growth, so far, has been controversial. For instance, IL-2, together with two other lymphokines, was needed for mouse B cell proliferation and differentiation (13); human B cells preactivated by SAC were shown to proliferate in response to purified IL-2, in accordance with our results (12). On the other hand, it has been reported (14, 15) that absorption of IL-2 from T cell–conditioned medium did not modify the proliferative response of activated normal B cells.

Although CLL B cells can differentiate, with T cell help, into plasma cells, we did not observe such an effect with IL-2. When IL-2-reponsive cells (four cases) were studied 3–12 d after SAC or anti-μ preactivation, the majority of cells were large immunoblasts devoid of cytoplasmic Ig.

These results raise important issues on the possible in vivo role of physiologic lymphokines such as IL-2 on the growth of the leukemic clone. It is worth noting that in one case, cells were responsive to IL-2 without any preactivation; in other instances, it is conceivable that antiidiotypic antibodies directed against surface Ig may trigger expression of IL-2 receptors, thus leading to possible regulation or expansion of the leukemic B cell clone by IL-2.

Summary

The proliferative responses of purified leukemic human B cells from nine B cell chronic lymphocytic leukemias to recombinant interleukin 2 (IL-2), spontaneously, and after preactivation by Staphylococcus aureus Cowan I (SAC) or anti-μ antibodies were studied. Three patterns of response were observed: (*a*) no response (three cases); (*b*) a moderate spontaneous response enhanced by anti-μ (one case); (*c*) a high proliferative response after preactivation by anti-μ and/or SAC (five cases). IL-2 could also trigger normal B cells, purified from spleen, to proliferative after preactivation by anti-μ or SAC. These results provide evidence that IL-2 is a lymphokine that acts physiologically on both B and T cells.

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