Antiproliferative Effects of Interleukin-4 on Freshly Isolated Non-Hodgkin Malignant B-Lymphoma Cells

By Thierry Defrance, Anne-Catherine Fluckiger, Jean-François Rossi, Jean-Pierre Magaud, Jean-Jacques Sotto, and Jacques Banchereau

The pattern of in vitro growth response of freshly isolated non-Hodgkin malignant lymphoma B cells (NHML) to cytokines was investigated. Ten tumor specimens of low- or intermediate-grade malignancy were selected for study. To assess their proliferative capacity in vitro, B-lymphoma cells were activated through ligation of their surface Ig receptor with insolubilized anti-IgM antibodies or Staphylococcus aureus strain Cowan I (SAC). In the great majority of cases, interleukin-2 (IL-2) was the sole factor that significantly and reproducibly stimulated DNA synthesis in NHML activated through their surface Iggs. Other B-cell tropic factors, including IL-4, IL-6, and tumor necrosis factor-α (TNF-α), failed to elicit a growth response in most of the IL-2-responsive neoplastic samples. However, one specimen among 10 exhibited the opposite pattern of response and proliferated following culture with IL-4 and anti-Ig reagents, but not after IL-2 stimulation. Three specimens could also be induced for DNA synthesis on cross-linking of their surface Iggs in the absence of exogenous growth factors. Although IL-4 could not support the in vitro growth of the majority of NHML cases, it strongly suppressed the proliferative signals delivered to these cells by anti-Ig reagents used alone or in combination with IL-2. Our data suggest that, in most cases, IL-4 essentially provides growth-inhibitory signals to NHML when they are activated through their surface Ig receptors and as such may be considered to be a valid candidate for future therapy of this type of mature B-cell malignancy.

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INTERLEUKIN-4 (IL-4) is a pleiotropic factor that has been shown to affect different steps of the antigen-dependent maturation process of human B cells. In quiescent B cells, IL-4 increases the expression of several functionally important surface antigens such as MHC class II determinants, CD23/FcεRγ, IgM, CD40, lymphocyte function-associated antigen (LFA)-1, and LFA-3. Following in vitro activation, IL-4 stimulates B-cell growth, as well as the production of IgG and IgM. Finally, IL-4 acts as a potent inducer of human IgE synthesis in the presence of T cells, after Epstein-Barr virus (EBV) stimulation, or following triggering of the CD40 antigens. Evidence has been provided that IL-4 could also deliver growth-inhibitory signals to B cells as exemplified by its capacity to antagonize the IL-2-dependent growth of normal B cells and of chronic lymphocytic leukemia B cells (B-CLL).

Although non-Hodgkin malignant lymphomas (NHML) and B-CLL are both classified as mature B-cell neoplasms, the immunohistologic characteristics, prognosis, and response to treatment of these two groups of B-cell malignancies are quite different. B-CLL are believed to represent the malignant expansion of a peculiar B-cell subset characterized by the constitutive expression of the pan-T-cell marker CD5 and localized at the edge of germinal centers in secondary follicles. According to their pattern of surface immunoglobulin expression (sIgM+, sIgD+), B-CLL are phenotypically related to mantle zone B cells. In contrast, the most frequent variant of NHML (centroblastic-centrocytic lymphoma) expresses some of the distinctive features of germinal center B cells (sIgD+, CD10+). Whereas the capacity of B-CLL cells to undergo proliferation in vitro in response to B-cell tropic factors has already been examined, the modulatory effects of cytokines on the in vitro growth of NHML are still poorly documented.

In this report, we show that NHML activated through their surface Ig receptors essentially proliferate in response to IL-2 and that IL-4 antagonizes their growth response to anti-Ig reagents used alone or in combination with IL-2.

MATERIALS AND METHODS

Isolation and purification of B-lymphoma cells. Pathologic samples were provided by Professor J.J. Sotto (Hospital Albert Michallon, Grenoble, France), Dr J.F. Rossi (Institute of Cancer-Val d’Aurelle, Montpellier, France), and Dr J.P. Magaud (Institute Edward Herriot, Lyon, France). Tumors with the diagnosis of non-Hodgkin, non-Burkitt, malignant lymphoma (NHML) according to the Working Formulation, who had not received chemotherapy for the 4 months preceding surgery, were selected for this study. The available specimens included six lymph nodes (EMZ, GAN, PP, MAI, PRO, VER) and four spleens (BOU, DEL, BRE, THE). Organs were cut with a scalpel blade and passed through a fine wire mesh to prepare a single-cell suspension. Mononuclear cells were obtained after centrifugation of the cell suspension over Ficoll/Hypaque gradient. For B-cell purification, mononuclear cells were first submitted to E rosetting with sheep red blood cells. The nonrosetting cells (E- fraction) were subsequently incubated with a cocktail of anti-T-cell (anti-CD3, anti-CD2) and antimonocyte (anti-CD14) monoclonal antibodies. Residual non-B cells were next removed from the E- population after incubation with magnetic beads (Dynabeads; Dynal, Oslo, Norway) coated with anti-mouse IgG.

Reagents. Insolubilized anti-IgM antibodies were purchased from Bio-Rad Laboratories (Richmond, CA) and were used at a final concentration of 10 µg/mL. Formalinized particles of Staphylococcus aureus strain Cowan I (SAC) were purchased as Pansorbin (EMZ, GAN, PP, MAI, PRO, VER) and four spleens (BOU, DEL, BRE, THE). Organs were cut with a scalpel blade and passed through a fine wire mesh to prepare a single-cell suspension. Mononuclear cells were obtained after centrifugation of the cell suspension over Ficoll/Hypaque gradient. For B-cell purification, mononuclear cells were first submitted to E rosetting with sheep red blood cells. The nonrosetting cells (E- fraction) were subsequently incubated with a cocktail of anti-T-cell (anti-CD3, anti-CD2) and antimonocyte (anti-CD14) monoclonal antibodies. Residual non-B cells were next removed from the E- population after incubation with magnetic beads (Dynabeads; Dynal, Oslo, Norway) coated with anti-mouse IgG.

Antibodies. The monoclonal and polyclonal antibodies used for the phenotyping of the B-lymphoma cell preparations were pur-
chased from the following manufacturers: Becton Dickinson Monoclonal Center (Mountain View, CA), Leu-1 (CD5), Leu-12 (CD19), Leu-16 (CD20), Leu-M3 (CD14), CD10, and HLA-DR; Immunotech (Luminy, France), IOT3 (CD3), IOT11 (CD2), and IOT14 (CD25); Dako (Glostrup, Denmark), fluorescein isothiocyanate (FITC)-conjugated P(ab)², fragments of polyconal anti-IgM, IgD, IgA, and IgG antibodies; Kallestad (Austin, TX); FITC-conjugated P(ab)², fragments of polyconal anti-human λ and κ light chain antibodies; Bioart (Meudon, France), polyconal goat antiserum against mouse IgG and IgM coupled to FITC used for indirect immunofluorescence stainings. The anti-CD40 monoclonal antibody 89 was prepared in the laboratory, as described elsewhere.¹⁹

The monoclonal antibodies used for the negative selection of B cells with magnetic beads were purchased from the following manufacturers: Aster Laboratories (La Gaude, France): anti-CD2 and anti-CD3; Immunotech: anti-CD14.

Cell staining was performed as described previously¹ and samples were analyzed with a fluorescence-activated cell sorter FACSCan (Becton Dickinson, Sunnyvale, CA). Negative controls for the immunofluorescence analysis were performed with the following antibodies: IgG₁ and IgG₂ controls (Becton Dickinson) for all the mouse monoclonal antibodies; a purified FITC-conjugated rabbit anti-interferon gamma (IFN-γ) antibody (prepared in the laboratory) for the anti-IgM, anti-IgD, and anti-IgG antibodies; a purified donkey anti-mouse IgM antibody (Pierce, Rockford, IL) for the donkey anti-λ antibody; a FITC-conjugated goat anti-mouse IgA antibody (Bioart) for the goat anti-κ antibody.

Propidium iodide (2 μg/mL) was added in each sample before flow cytometry analysis to gate-out nonviable cells.

Ki 67 immunostaining. Purified NHML cells (1 × 10⁵ cells under a final volume of 200 μL in round-bottomed microtiter trays) were cultured for 4 days in complete medium under various conditions of stimulation. Positive controls were performed with a lymphoblastoid cell line harvested in its logarithmic phase of growth. Cells were harvested and cytocentrifuged. The slides were then air-dried and fixed for 1 minute in acetone. The Ki 67 immunostaining procedure was performed using a labeled streptavidin biotin kit purchased from Dako (Santa Barbara, CA) according to the experimental protocol recommended by the manufacturer. Briefly, the slides were successively incubated with the anti-Ki 67 murine monoclonal antibody (Dako, Glostrup, Denmark) or with an isotype matched nonrelated mouse anti-IgM antibody prepared in the laboratory) and with the biotinylated goat anti-mouse antibody. Staining was shown using peroxidase-conjugated streptavidin. The counterstaining was performed with Mayer’s hematoxylin solution (Sigma, St Louis, MO). Results were expressed as percentages of Ki 67-positive cells.

Factors. Purified recombinant IL-1α (1 × 10⁶ U/mg) was purchased from Genzyme (Boston, MA), whereas IL-3 and GM-CSF were used at concentrations of 10 ng/mL and 100 ng/mL, respectively, which were determined to be saturating for the growth and differentiation of hematopoietic precursors in liquid cultures or in colony assays performed in semisolid medium. Purified recombinant human IL-4 (derived from Escherichia coli, 1 × 10⁸ U/mg) was provided by Drs P. Trotta and T.L. Nagabhushan (Schering-Plough Research). In most of the experiments, IL-4 was used at the final concentration of 500 U/mL, which provides maximal stimulation of B-cell growth, as estimated on normal B cells activated with insolubilized anti-IgM antibodies. Purified tumor necrosis factor (TNFα; Genzyme) was used at the final concentration of 25 ng/mL, which was determined to provide the plateau of [³H]thymidine ([³H]TdR) incorporation in normal B cells costimulated with insolubilized anti-IgM antibodies. Purified recombinant human IL-6 (Genzyme) was used at five times the optimal concentration determined in the specific IL-6 biological assay using the murine plasmacytoma cell line KD83 (obtained through the courtesy of Dr F. Lee, DNAX).

Cultures. Purified B-lymphoma cells were cultured in Iscove’s medium (Flow Laboratories, Irvine, CA) enriched with 50 μg/mL human transferrin (Sigma), 0.5% bovine serum albumin (Sigma), 5 μg/mL bovine insulin (Sigma), 5% selected heat-inactivated fetal calf serum (Flow Laboratories), 100 U/mL penicillin (Flow Laboratories), 100 μg/mL streptomycin (Flow Laboratories), and 10⁻⁶ M of β-mercaptoethanol (Sigma). For proliferation assays, B-lymphoma cells were plated at 1 × 10⁵ in 100 μL of culture medium in round-bottom microcells and incubated in a 5% CO₂, humidified atmosphere at 37°C. Polyclonal B-cell activators (SAC, and insolubilized anti-IgM antibodies) and factors were added at the onset of the culture at the concentrations listed above. DNA synthesis was determined by pulsing cells with [³H]TdR for the final 16 hours of the culture period. Due to the heterogeneity of the responses of B-lymphoma cell samples, DNA synthesis was assessed at four different time intervals (days 3, 4, 5, and 6) after onset of the culture. The results presented correspond to the time point that provided the maximal stimulation indices. Counts of [³H]TdR incorporation are expressed as means of triplicate determinations. In preliminary experiments, the harvesting of two identical culture plates was performed in presence or absence of trichloroacetic acid (TCA). Since the tritium counts were strictly comparable in both cases, we assumed that the [³H]TdR uptake was incorporated into DNA. In certain experiments in which the viable cell recovery under various culture conditions had to be determined, 1 × 10⁶ purified B-lymphoma cells were seeded in round-bottomed microtiter trays, under a final volume of 100 μL, each culture point being performed in pentaplicates.

RESULTS

Phenotypic characteristics of the NHML samples. Data summarized in Table 1 show that T cells and monocytes were virtually absent from the purified B-cell preparations, since no positive staining was observed with anti-CD2, anti-CD3, and anti-CD14 monoclonal antibodies. Accordingly, in most cases, the cells homogenously expressed the pan-B-cell markers CD19 and CD20, as well as the HLA-DR antigens with a monotonal fluorescence histogram peak clearly separated from that of the control. Most of the lymphoma specimens (9 of 10) were found to express sIgM, whereas sIgD was less represented, since cells from only five patients (DEL, GAN, MAI, PP, and PRO) expressed significant levels of this antigen. One leukemic sample (BOU) expressed sIgG. All B-cell populations isolated from lymphoma samples were found to exclusively
express only one type of light chain, therefore arguing in favor of the monoclonality of the studied populations and ruling out the presence of significant numbers of normal B cells. A significant proportion of cases (five of 10) expressed CD10 (CALLA), as previously reported. Some of the tumor cells (four of 10) were also found to bear the CD5 antigen.

IL-2 stimulates the in vitro growth of NHML activated through their sIg receptors. To determine the pattern of in vitro reactivity of NHML to cytokines, four different purified B-lymphoma specimens (BRE, GAN, BOU, and PP) were assessed for DNA synthesis in response to costimulation with anti-Ig reagents (SAC and insolubilized anti-IgM) and the following cytokines: IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, GM-CSF, IFN-γ, and TNF-α. Factors and reagents were added to cultures at concentrations that had previously been defined as optimal in specific bioassays, as described in the Materials and Methods. Figure 1 illustrates the results of one representative experiment in which the lymphoma specimen PP was costimulated with SAC and each of the factors listed above for a culture period of 4 days. In the absence of in vitro activation, none of the factors stimulated [3H]TdR incorporation above background levels (data not shown). In the presence of anti-Ig reagents, the tumor cells were essentially stimulated for DNA synthesis by IL-2, although some marginal responses could also be obtained with IFN-γ in certain cases. Apart from the tumoral clone BRE, which was moderately stimulated for DNA synthesis by IL-4, most NHML specimens were not induced for [3H]TdR incorporation on costimulation with IL-4 and anti-Ig reagents (Table 2). This latter finding is in striking contrast with the well-documented growth-promoting effect of IL-4 on normal human B cells.1 The effects of IL-2 used in combination with SAC or anti-IgM antibodies on the in vitro growth of the 10 neoplastic samples studied are presented in Table 3.

Three samples (PRO, EMZ, BOU) displayed a high proliferative response to the anti-Ig reagents (SAC for BOU and SAC and anti-IgM antibodies for PRO and EMZ) in absence of exogenous growth factors. Addition of IL-2 further enhanced the growth response of tumoral B cells from patient EMZ to anti-IgM antibodies, and potentiated the signal delivered by SAC to B cells from patient BOU. Five lymphoma samples (GAN, PP, DEL, THE, and VER) were only moderately stimulated for DNA synthesis following cross-linking of their surface Igs by SAC or anti-IgM antibodies, but proliferated significantly when costimulated with anti-Ig reagents and IL-2. Finally, two specimens (BRE and MAI) were found to be only marginally responsive to IL-2 whatever the activating agent used. The heterogeneity observed in the activation requirements

### Table 1. Phenotypic Profile of Purified B-Lymphoma Cells From NHML Patients

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Cell surface markers were analyzed after depletion of non-B cells from the lymphoma samples.

Abbreviation: ND, not determined.

*Histological diagnosis according to the Working Formulation: FML, follicular mixed lymphoma; FSCL, follicular small cleaved cell lymphoma; DSCL, diffuse small cleaved cell lymphoma; SLL, small lymphocytic lymphoma.

†No detectable positive staining, the fluorescence histogram was superimposed with that of the negative control.

‡One hundred percent of positive cells means that the staining histogram was monotonal and clearly separated from that of the negative control.

§IgM and IgD heavy chains and λ light chains were expressed on clone DEL, but with a very low staining intensity.
of the tumor specimens was taken into account in further experiments in which each lymphoma sample was activated with the appropriate anti-Ig reagent to provide the optimal response to the growth-stimulatory effect of IL-2.

IL-4 antagonizes the IL-2-dependent growth of NHML. Given the previously described inhibitory effects of IL-4 on IL-2-mediated growth of normal B cells and B-CLL, IL-4 was assayed for its capacity to antagonize the proliferative response of NHML to IL-2. Each of the 10 tumor specimens of NHML was therefore cocultured with IL-2 (20 U/mL) and IL-4 (500 U/mL) in the presence of the anti-Ig reagent previously defined as being optimal for activation. Since the lymphoma samples also differed from one another in terms of time kinetics of the response to the growth-promoting effect of IL-2, DNA synthesis was measured 4, 5, 6, and 7 days after onset of the culture. Data displayed in Table 2 represent, for each specimen, the levels of [\(^{3}H\)Tdr] incorporations obtained at the time point corresponding to the peak of the proliferative response to IL-2. One tumoral sample (MAI) did not display significant growth response to IL-2 or IL-4 in both activation systems and therefore is not included in the table.

In eight of nine cases, IL-4 failed to synergize with antiimmunoglobulins reagents to support DNA synthesis from NHML. With the exception of B cells from patient BRE, all NHML samples were stimulated for DNA synthesis after costimulation with antiimmunoglobulin reagents and IL-2, although some heterogeneity was observed in terms of magnitude of the proliferative response. For all IL-2-responsive specimens, IL-4 was found to significantly inhibit the IL-2-driven proliferation of the cells. The titration of the growth-inhibitory effect of IL-4 on the IL-2-induced response of the B-lymphoma cells from patient GAN activated with SAC (Fig 2) shows that complete inhibition of the response to IL-2 is already achieved for concentrations of IL-4 as low as 16 U/mL. Moreover, IL-4 also inhibited the growth of the three tumor specimens (BOU, EMZ, and PRO) that already exhibited a significant proliferative response after ligation of their surface immunoglobulins with SAC or anti-IgM antibodies (Table 2, Fig 3). The statistical analysis of the variations of the [\(^{3}H\)Tdr] uptake values observed in the various culture conditions mentioned above was performed using the Wilcoxon rank test (we accepted a P value of .05 as indicating statistical significance). It permitted establishment of the general pattern of growth response of NHML to IL-2 and IL-4: (1) anti-Ig-activated B-lymphoma cells are stimulated for DNA synthesis by IL-2, the difference between the values obtained with polyclonal B-cell activator (PBA) and PBA + IL-2 being significant (P < .05); and (2) IL-4 counteracts the growth-stimulatory effect of IL-2 on anti-Ig-activated B-lymphoma cells, the difference between the values obtained with PBA and PBA + IL-2 + IL-4 being significant (P < .05).

We next examined whether IL-4 was inhibiting cellular replication or only a process of thymidine uptake unrelated to cell proliferation. For this purpose, the viable cell recovery, as well as the percentage of Ki 67-positive cells, was estimated for four different NHML specimens after 4 days of culture with the following stimuli: medium, SAC, SAC + IL-2, SAC + IL-4, SAC + IL-2 + IL-4. The results of one representative experiment performed on the tumoral clone VER are illustrated in Fig 4. The cell counts and the Ki 67 data were in agreement with the results previously obtained in the [\(^{3}H\)Tdr] incorporation assay. Indeed, IL-2 enhanced both the numbers of viable cells recovered and the proportion of Ki 67-positive cells, whereas IL-4 failed to display any stimulatory effect in both assay

| Table 2. IL-4 Antagonizes the IL-2-Dependent Growth of NHML |
|------------------|---------|------|------|-------|------|------|------|------|------|------|
|                  | SAC     | Anti-IgM |
| Medium           | 0.5     | 0.3    | 0.2  | 0.9   | 7.1  | 1.2  | 0.2  | 0.3  | 0.2  |
| PBA              | 21.4    | 2.3    | 6.2  | 6.2   | 4.6  | 0.6  | 18.2 | 64.8 | 2.4  |
| PBA + IL-4       | 11.9    | 2.0    | 3.2  | 5.3   | 6.5  | 1.6  | 7.8  | 30.1 | 9.1  |
| PBA + IL-2       | 35.5    | 7.9    | 47.2 | 21.6  | 46.2 | 8.4  | 56.8 | 72.3 | 4.5  |
| PBA + IL-2 + IL-4| 12.8    | 3.9    | 9.6  | 6.7   | 8.3  | 3.7  | 13.6 | 27.8 | 11.1 |

Cells (1 x 10^6) from each B-lymphoma specimen were cultured for 4 days with SAC (0.005%) or insolubilized anti-IgM antibodies (10 μg/mL) used alone or in combination with IL-2 (20 U/mL). Results (cpm x 10^-1) are expressed as means of triplicate determinations, which never varied by greater than 10%.

| Table 3. The Proliferative Response of NHML to Anti-Ig reagents and IL-2 is Heterogeneous |
|------------------|-------|-----|-----|-------|------|------|------|------|------|------|
|                  | PRO   | EMZ | BOU | GAN  | PP   | DEL | THE | VER  | BRE  | MAI  |
| Medium           | 0.3   | 0.2 | 0.3 | 0.6  | 0.2  | 0.9 | 0.3 | 7.1  | 0.2  | 1.1  |
| SAC              | 146.6 | 86.8| 12.5| 4.6  | 6.2  | 4.4 | 2.3 | 4.6  | 0.6  | 0.6  |
| SAC + IL-2      | 140.1 | 101.3| 44.6| 11.9 | 47.2 | 28.9| 7.9 | 47.1 | 2.9  | 1.8  |
| Anti-IgM         | 57.6  | 18.2| 0.2 | 0.4  | 0.9  | 0.7 | 1.0 | ND   | 2.3  | 0.5  |
| Anti-IgM + IL-2 | 67.7  | 56.8| 1.0 | 8.0  | 12.6 | 9.2 | 3.3 | ND   | 4.5  | 0.5  |

Abbreviation: PBA, polyclonal B-cell activator.
Dose-response curve of the inhibitory effect of IL-4 on the IL-2-driven proliferation of B cells from patient GAN. 1 × 10^5 cells of the tumor sample GAN were costimulated with SAC (0.005%) and IL-2 (10 U/mL) in the absence or in the presence of serial dilutions of IL-4. [3H]Tdr incorporation was assessed at day 4. The histogram bars represent the proliferative response of the lymphoma cells to IL-4 alone, and the curve represents the proliferative response of the lymphoma cells cocultured with IL-2 and serial dilutions of IL-4. Representative of three experiments.

**DISCUSSION**

In the present study, we show that NHML activated through their surface Ig receptors can be stimulated for DNA synthesis by IL-2. In some tumor specimens (three of 10), a strong proliferative response could also be induced by the sole ligation of the surface Igs in absence of exogenous growth factors. In agreement with previously reported data on B-CLL, IL-4 failed to display any growth-promoting activity on most of the NHML samples, but significantly suppressed not only the growth response of these cells to IL-2, but also to the anti-Ig reagent by itself. The observation that IL-2 increases the numbers of Ki 67-positive cells and enhances the viable cell recovery suggests that IL-2 stimulates cellular replication at least in a fraction of the B-cell population examined. Two lines of evidence argue against the possibility that this pool of IL-2-responsive cells could entirely consist of residual normal B cells. First, whatever the stimulus applied, the B-lymphoma cell suspensions exclusively expressed the surface Ig phenotype (heavy and light chains) identifying the neoplastic clone on tissue sections, even after prolonged culturing (data not shown), therefore supporting the notion that the B-cell population studied originates from the tumor. Second, considering that more than 25% of the cells in some B-lymphoma specimens could be induced to express the Ki 67 antigen and that the sIg phenotype after culture is homogenous, it is likely that the majority of cycling cells are of neoplastic origin. Moreover, since IL-4 antagonized not only the stimulatory effect of IL-2 on [3H]Tdr uptake, but also the IL-2-mediated enhancement of Ki 67 expression and viable cell recovery, we assume that IL-4 truly inhibits the cellular replication of the B-lymphoma cells.

The relationship between the in vitro responsiveness of NEML to IL-2 and the mechanism that governs expansion of the B-lymphoma cells in vivo remains an unresolved
issue. IL-2 could be produced locally by activated T cells present at the tumor site or by the neoplastic B cells themselves, possibly following interactions with the microenvironment. However, our observation that IL-4 could antagonize the anti-Ig-mediated proliferation of three lymphoma specimens would suggest that the growth-inhibitory effect of IL-4 on malignant B cells might not be restricted to the proliferative signal delivered by IL-2, but could rather affect some general mechanism involved in B-cell tumor growth. In agreement with this hypothesis, Taylor et al. have recently reported that IL-4 could inhibit the spontaneous in vitro growth of a wide variety of lymphoid neoplasms, including aggressive types of diseases such as diffuse lymphoblastic lymphomas and multiple myelomas.

Among the various cytokines tested for induction of proliferation of NHML, only IL-2 displayed a reproducible and significant growth-promoting activity on the panel of lymphoma specimens we have studied. However, Yee et al. have described the establishment of cell lines derived from NHML that were dependent on IL-6 for their growth. The lack of stimulatory effect of IL-6 on NHML in our hands may partly relate to the clinical stage of the group of patients studied. Indeed, our panel of NHML samples mainly consisted of low- and intermediate-grade malignant lymphomas, whereas the IL-6-dependent lymphoma cell lines described by Yee et al. originated from high-grade malignant lymphomas. Alternatively, it can be postulated that there could exist some heterogeneity in the nature of the factors required for tumor growth, even within a given pathology. Our observation that one tumor specimen (BRE) was stimulated for DNA synthesis by IL-4 and not by IL-2 in co-stimulation with Ig cross-linking agents would argue in favor of such a hypothesis. However, a more extensive study would be required to define whether responsiveness to the growth-promoting effect of IL-4 in NHML could be associated with a peculiar stage of the disease or to a given stage of normal B-cell maturation represented by some rare cases of lymphomas. It should be noted that the maximal inhibitory effect of IL-4 on IL-2 responsiveness of NHML is obtained for doses of purified recombinant IL-4 ranging from 10 to 100 U/mL, which have also been shown to provide the maximal suppression of the IL-2-driven proliferation of normal B cells and B-CLL. This observation is consistent with the notion that NHML and normal B cells are identical in terms of affinity or functionality of the IL-4 binding sites involved in the transduction of the growth-inhibitory signal delivered by IL-4. Therefore, considering that neoplastic B cells (NHML and B-CLL) activated through their surface IgS, differ from normal B cells by their inability to proliferate in response to IL-4, it is tempting to speculate that the agonistic and antagonistic effects of IL-4 on B cells can be uncoupled.

Several lines of evidence now suggest that IL-4 could display antitumor activity: either by activating antigen specific cytotoxic T cells as demonstrated by the studies of Widmer et al. or by activating macrophages and eosinophils as recently suggested by Tepper et al. by directly suppressing the growth of the tumor cells themselves as suggested by our data. Taken together, these results provide the rationale for examination of IL-4 as a potential therapeutic agent in the treatment of mature B-cell malignancies.

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Antiproliferative effects of interleukin-4 on freshly isolated non-Hodgkin malignant B-lymphoma cells

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