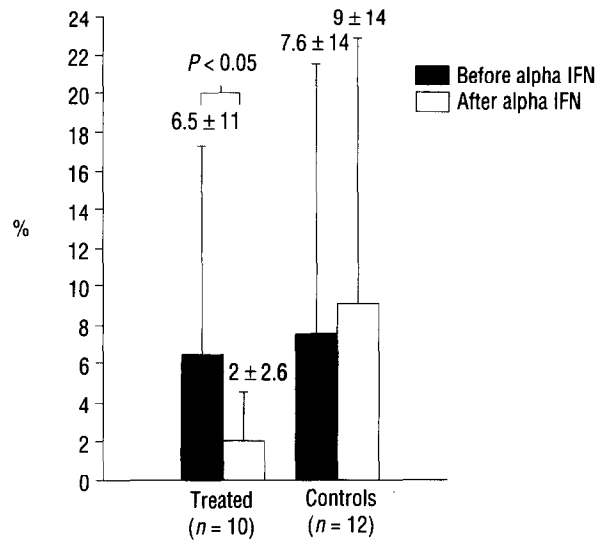

Fig. 3. Serum cryocrit levels.

pyruvic transaminase (SGPT) levels ($P < 0.05$) (Fig. 2), and in serum cryocrit ($P < 0.05$) (Fig. 3). Patients receiving alpha interferon also showed a significant improvement in CD4/CD8 ratio ($P < 0.025$); in half of the patients, this parameter was below the normal range at the beginning and was normalized after 1 month of treatment. After alpha interferon, anti-HCV antibodies disappeared in three of five patients ($P < 0.001$), while in the control group they remained unchanged (Table 1).

Drop out

Only one of the 10 patients treated with alpha interferon dropped out, after a 3-week period, because of worsening peripheral neuropathy.

CONCLUSIONS

Alpha interferon positively affected clinical manifestations of MC, such as severity and frequency of purpura, and signs of liver involvement (increased SGPT levels) that were present in 60% of cases. These results were mirrored by changes in immunological parameters. Although preliminary, these results suggest that alpha interferon may be considered an interesting therapeutic approach, able to affect, at different levels, the pathogenetic mechanism(s) of MC and improve some clinical manifestations of the disease.

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Molecular Events in B-Cell Activation and Growth: Sensitivity to Alpha Interferon

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INTRODUCTION

IN ORDER TO determine molecular targets for alpha interferon and other agents on B-cells, we have studied important molecular events in normal peripheral B-cell activation and growth. The purpose was to identify interferon-sensitive events as a platform for further studies on interferon-sensitivity of B-cell tumours.

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MATERIALS AND METHODS

Isolation of primary B-cells

Buffy coats were obtained from healthy donors. The isolation process consisted of lymphoprep separation of mononuclear cells, anti-CD19 immunomagnetic beads, and complement lysis of T-cells. The final cell population contained less than 0.1% CD2 positive cells.

Growth of B-lymphocytes

B-lymphocytes were grown with anti-IgM antibodies, phorbol ester (PDB)/Ca-ionophor (ionomycin), cocultivation with lymphokines, and membranes from activated T-cells.

RESULTS

Effect of mitogenic factors on growth parameters

In spite of significant stimulation of thymidine uptake after 3 days, cell numbers did not increase (Table 1).

Table 1. Effect of mitogenic factors on growth parameters

Mitogenic factor	cpm	Cells/ml
Medium control	45	18,000
Anti-IgM (3 μg/ml)	177	108,000
Anti-IgM, BCGF (10%)	696	108,000
Anti-IgM, BCGF, IL-2 (20 U/ml)	1,885	126,000
PDB (5 ng/ml), ionomycin (2 μg/ml)	5,754	102,000

Input cell number: 200,000 cells/ml.

Cytofluorographic analysis of cellular DNA content after growth in the presence of BUDR (Fig. 1) revealed that after

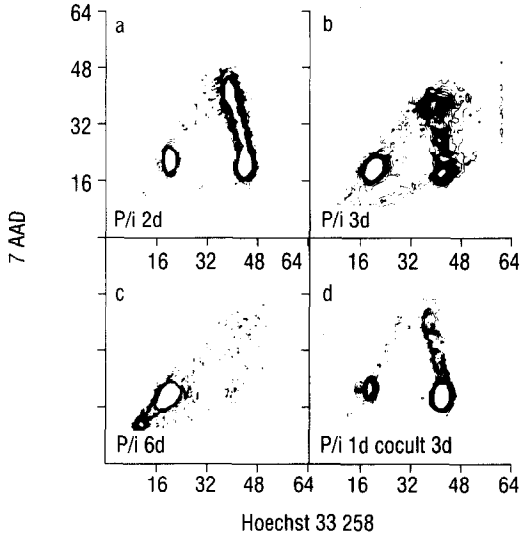

Fig. 1. Cytofluorographic analysis of cellular DNA content double-stained with 7-AAD (10 μg/ml) after growth in the presence of BUDR. a) PDB/ionomycin treatment for 2 days (cells remained in first cycle); b) PDB/ionomycin for 3 days (most cells had left first cycle but seemed to be arrested in G_1 of second cycle); c) PDB/ionomycin for 6 days; d) PDB/ionomycin for 1 day followed by growth under cocultivation conditions in anti-IgM and BCGF, IL-2 and IL-4 (cells have initiated both a second and third cycle).

PDB/ionomycin treatment for 2 days, the cells remained in the first cycle. After PDB/ionomycin for 3 days, most of the cells had left the first cell cycle but seemed to be arrested in G_1 of the second cycle. Cells grown under PDB/ionomycin for 1 day followed by cocultivation conditions in anti-IgM and BCGF, IL-2 and IL-4 for 3 days, were shown to have initiated both a second and third cycle.

Effect of alpha interferon on B-cell stimulation

The effect of alpha interferon on B-cell stimulation is shown in Table 2. The proliferative response was only inhibited by alpha interferon when B-cells were activated by PDB/ionomycin, and not by cocultivation with anti-IgM,

Table 2. Effect of alpha interferon on B-cell stimulation

Treatment	Control	Alpha IFN (1000 U/ml)
Medium	313 ± 243	ND
Anti-IgM, BCGF, IL-2	1,481 ± 114	2,932 ± 147
PDB, ionomycin	10,631 ± 710	4,382 ± 157

ND = not done.

BCGF and IL-2. Cells activated in the presence of alpha interferon were found to be less sensitive to IL-2 (data not shown).

Tac-antigen expression

Tac-antigen expression of B-cells activated by PDB/ionomycin for 24 hours and grown in BCGF and IL-2 was found to be reduced by exposure to alpha interferon (Fig. 2),

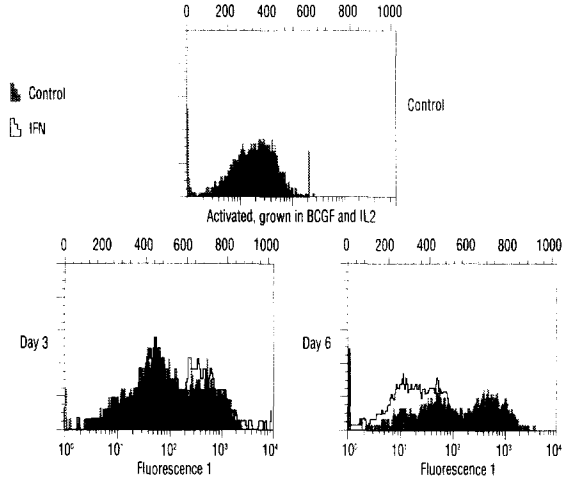

Fig. 2. Tac-antigen expression of B-cells activated by PDBu (5 ng/ml) and ionomycin (2 μg/ml) for 24 hours and grown in BCGF (10%) and IL-2 (20 U/ml). Tac-antigen expression was measured cytofluorometrically, and percentages of Tac-positive cells were arbitrarily defined as cells giving a signal stronger than the bar shown in the medium control panel.

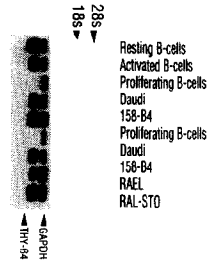

Fig. 3. Expression of thymosin-ß4 in primary B-cells, some EBV-immortalized cell lines (158-B4, RAEL-STO) and Burkitt's lymphoma cells (Daudi and RAEL).

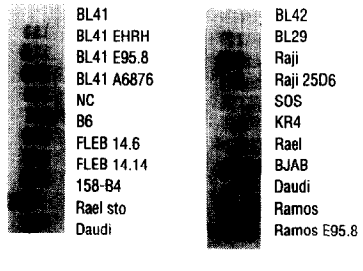

Fig. 4. Expression of EBV77, isolated from a cDNA library from an EBV-immortalized B-cell line, in a panel of B-cell lines. Three transcripts of 1.5 kb, 1.2 kb and 0.9 kb were found, the smallest being differentially expressed. BL41 and Ramos are EBV-negative Burkitt's lymphoma cell lines, which have been superinfected with different EBV isolates.

from 33.2% Tac-positive cells on day 3 to 3.4% on day 6. There was no change in the percentage of Tac-positive cells in the untreated control sample (42.9% on day 3, versus 46.1% on day 6).

Screening of cDNA libraries

Several genes have been isolated by screening cDNA libraries from resting and growing B-cells, as well as from Epstein-Barr virus (EBV)-transformed B-cell lines, including the interferon-inducible gene thymosin-ß4 (Fig. 3) and the gene designated EBV77 (Fig. 4), which is unique and not represented in GenBank.

CONCLUSION

These studies showed that human peripheral B-cells could be activated to growth either by phorbol ester and Ca-ionophor or by a cocultivation protocol using anti-IgM and several lymphokines. The first protocol was alpha interferon-sensitive and associated with loss of Tac-antigen expression but not the second. Several genes have been isolated from a cDNA library derived from growing B-cells, of which one was expressed with three transcripts, differentially regulated by EBV.

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The Use of Interferon in Renal Cell Carcinoma

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Metastatic renal cell carcinoma remains an incurable disease and current modalities can only offer major palliation to a small percentage of patients. Since treatment is palliative, choice and type of therapy must be carefully considered and reconciled with patient desires. When possible, patients should be offered participation in a clinical trial. For patients choosing progestin therapy, treatment with interferon (IFN) or other biological response modifiers can be instituted at the time of progestin failure. Those patients who have slow tumour progression and maintain a high quality of life can be observed without continued progestin therapy. Although pretreatment characteristics predict response to biologicals, no pretreatment characteristic should preclude an individual patient from a trial of IFN therapy. Whether high-dose interleukin-2 (IL-2), IL-2/lymphocyte-activated killer cells, or IL-2/IFN are superior to IFN alone is uncertain, but clinical trials currently underway should help resolve these issues.
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INTRODUCTION

THE AMERICAN CANCER SOCIETY estimates that there will be almost 24,000 cases of renal cell carcinoma in the United States in 1990 resulting in over 10,000 deaths [1]. Although the aetiology of renal cell carcinoma is unknown and there is no effective screening strategy, approximately 50% of patients present with localized disease amenable to surgical cure. Patients with recurrent or metastatic disease have a poor prognosis with a mortality rate of 74% at 1 year and 96% at 3 years [2]. The most common metastatic sites include the lung (65% of patients), bone (40%), liver (14%), adrenals (8%), and peritoneum (8%) [2]. The phenomenon of spontaneous regression seen in almost 1% of patients [3, 4] and the array of paraneoplastic syndromes [5] including fever, cachexia, polycythaemia or anaemia, hypercalcaemia and hepatitis, all associated with renal carcinoma, suggest that several biological substances are secreted by these tumours. The following review will focus on the treatment of advanced or recurrent disease with biological therapy, mainly the interferons. In addition, the role of endocrine therapy and chemotherapy will be discussed.

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HORMONAL THERAPY AND CHEMOTHERAPY

Progestin therapy remains a popular modality in patients with metastatic disease. Original reports by Bloom [6] indicated a response rate with progestin of 16%, but a more recent review by Hrushesky and Murphy [7] suggests that the true objective response to endocrine therapy is less than 5%. Nevertheless, their ease of use, minimal side effects and potentially beneficial